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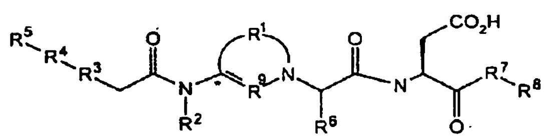
(54) Title: MODIFIED PENTAPEPTIDE ANTAGONISTS OF THE ATRIAL NATRIURETIC PEPTIDE CLEARANCE RECEPTOR



(A)

(57) Abstract

A compound having general formula (A) and methods of using such compounds for the treatment of diseases and pharmaceutical composition comprising such compounds.

Applicants: Kiran K. Chada et al.
U.S. Serial No. 10/768,566
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Exhibit 1

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MODIFIED PENTAPEPTIDE ANTAGONISTS OF THE ATRIAL NATRIURETIC PEPTIDE CLEARANCE RECEPTOR

Background

ANP is a member of a family of natriuretic peptide hormones, which includes atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP). The natriuretic peptides have a number of actions on the cardiovascular system, including; natriuresis, diuresis, and relaxation of vascular smooth muscle. ANP is a 28-amino acid cyclic peptide which is produced in atrial myocytes in response to increases in heart rate and atrial stretch.

There are two biologically- and functionally-distinct classes of ANP receptors. The first one is linked to guanylate cyclase and is thought to mediate the physiological effects of ANP via increases in intracellular cGMP levels. These guanylate cyclase receptors are further divided into the ANP-A and ANP-B receptors according to their relative affinity for different natriuretic peptides. The second class of ANP receptors do not mediate the cardiovasculature effects of the hormone and are thought to mainly serve a clearing function of ANP from the extracellular circulation. This receptor is known as the atrial natriuretic peptide clearance receptor (ANPCR).

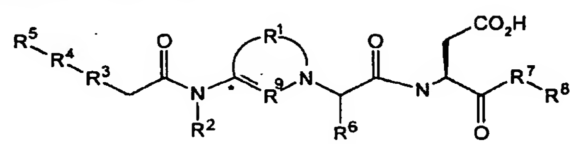
Natriuretic peptides have a very short half life *in vivo*, and there are thought to be two major modes of their clearance from systemic circulation. One is via proteolytic inactivation by the enzyme neutral endopeptidase (NEP). The other is via binding to the ANP clearance receptor which is expressed on the vascular endothelium. Binding to the clearance receptor is followed by internalization and degradation of the peptide. The lung is thought to play a major role in ANP clearance, and studies have found over 50% of ANP is cleared in a single pass through the lungs. Approaches based on the inhibition of NEP are further complicated by the number of physiologically important peptide hormones which are substrates for this enzyme. Of the two modes of clearance, the ANPCR is thought to be primarily responsible for removal of ANP in the pulmonary vasculature, and the ANPCR is the dominate ANP receptor in lung tissue. Additionally, blockade of the clearance receptor in the lung was thought to provide a pulmonary selective approach to reduction of pulmonary blood pressure due to the presence of both the ANP-A,B and ANPCR receptors in the lung and by the proximity of these receptors to the site of ANP synthesis. For these reasons blockade of the ANPCR was chosen as the best approach to increase endogenous levels of ANP.

It is estimated that a large segment of patients with chronic obstructive pulmonary disease ("COPD") will develop pulmonary hypertension (> 6 million in the US alone), and that the number of patients will increase as diagnostic methods improve. An ANPCR antagonist could have therapeutic usefulness in treating pulmonary hypertension secondary to COPD. In addition, since all natriuretic peptides (i.e., ANP, BNP and CNP) inhibit vascular smooth muscle cell proliferation, an ANPCR antagonist may also be useful for protection of the transplanted heart given that plasma levels of BNP are elevated in this situation. An ANPCR antagonist may have the greatest therapeutic utility in the treatment of congestive heart failure (CHF), by virtue of raising plasma concentrations of ANP and BNP. Infusion of exogenous BNP decreased plasma renin activity, increased plasma cGMP and increased urinary sodium output with concomitant decreases in pulmonary capillary wedge pressure in the dog model of acute heart failure. It has been hypothesized that ANP and BNP's role in the circulation may be to produce venodilation and increase capillary permeability to reduce cardiac preload and prevent pulmonary congestion. Increased plasma ANP and BNP would be expected by blockade of the ANPCR receptor.

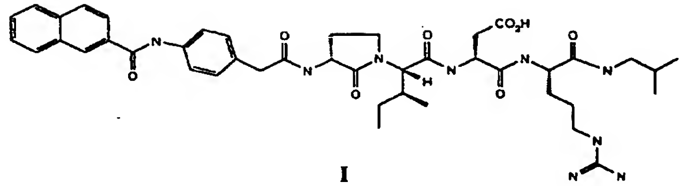
Summary of the Invention

The present invention is directed to synthetic analogs of atrial peptides and more particularly o synthetic linear peptide analogs which find use as diuretics, natriuretics and/or vasodilators, or as intermediates for or modulators of such useful compounds, together with methods for their production and use.

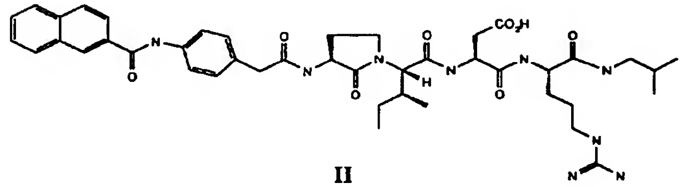
A series of lactams of the generic structure shown below were found to be blockers of the ANP clearance receptor (ANPCR).



Such a replacement group contains a chiral center at the lactam α-carbon, giving the diastereomeric pair **I** and **II**. It has now been found that the R-isomer of the lactam is preferred, as shown in the structures below.

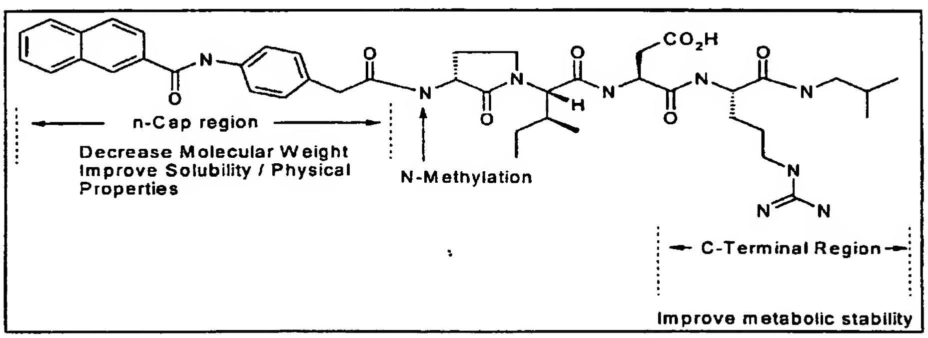


I

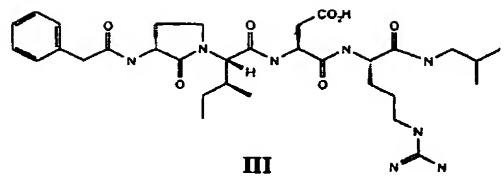


II

In the course of this work is was found that lactam molecules which contained a D-amino acid or a sarcosine residue in position R7 has good metabolic stability. This is in contrast to the natural hormone ANP which has poor metabolic stability.

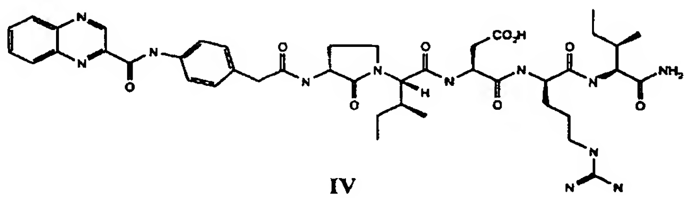


Work in the n-Cap region found that decreases in molecular weight caused large decreases in potency. For example, truncation to the phenylacetic carboxamide (III) resulted in complete loss of binding affinity, while more modest truncation of the naphthyl group to a series of substituted benzamides generally produced compounds that bind in the 100 nM range.



III

A number of promising compounds in terms of biological activity resulted from work in the n-Cap region. A series of heterocyclic replacements for the naphthyl group, were most promising. A compound which combined a 2-quinoxalazine as a replacement for the naphthyl ring, coupled with a D-Arg-14 residue (IV) shows promising oral activity.



IV

Brief Description of the Drawings

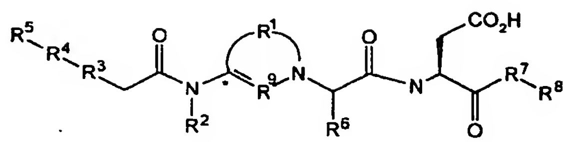
Some data related to the invention will now be described with reference to the accompanying drawings, in which:

Figure 1 is a chart showing the dose-response effect of **IV** or vehicle administered orally 165 min before evaluating the change in right intraventricular peak systolic pressure (RVSP; upper panel) and mean systemic arterial pressure (MAP; lower panel); and

Figure 2 is a chart showing the effect of vehicle or **I** (30 mg/kg, p.o.; top panel) and **IV** (100 mg/kg, p.o.; bottom panel) on immunoreactive plasma content of ANP in rats exposed to acute hypoxia.

Detailed Description of the Invention

The compounds of the instant invention are linear peptide compounds having the structure:



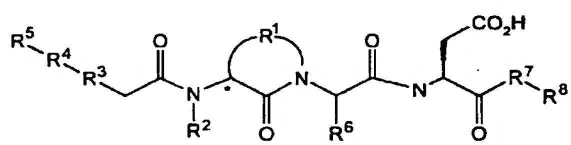
In this structure:

R^1 is a hydrocarbon chain containing from one to four carbon atoms and zero-to-two heteroatoms, but is preferably $-CH_2CH_2-$, $-CH_2CH_2CH_2-$, $=CH-CH=CH-$ or $-N=CH-$;
R^2 should be hydrogen or a C_1-C_4 alkyl group, but is preferably hydrogen or methyl. R^3 is a zero-to-four atom chain or aromatic ring containing from zero-to-eight carbon atoms and zero-to-three heteroatoms;
R^3 is preferably $-CH_2CH_2CH_2-$, -(E)-CH=CHC(=O)NH-, $-CH_2CH_2C(=O)NH-$, para-disubstituted phenyl, ortho-disubstituted phenyl, meta-disubstituted phenyl or a single bond, wherein, in the disubstituted phenyl groups, one substituent is R^4 and the other is the methylene group alpha to the amide carbonyl, as shown in the generic structure above;
R^4 is -NHC(=O)-, -C(=O)NH- or $-S(=O)_2NH-$;
R^5 is a substituted or unsubstituted alkylaryl, aryl or heteroaryl compound, preferably 1-naphthyl, 2-naphthyl, $-CH_2CH_2NHCH_2CH=CH$-phenyl, $-CH_2CH_2$-phenyl, -CH=CH-phenyl, 2-quinolyl, 3-quinolyl, 4-quinolyl, 6-quinolyl, 3-isoquinolyl, 2-quinoxaline, 5-chloro-2-indolyl, 2-indolyl, 4-chlorophenyl, 4-methylphenyl, 3-methoxyphenyl, 4-cyanophenyl, 3,4-difluorophenyl, 3-chloro-4-fluorophenyl, 2,4-dichlorophenyl, 3,4-dichlorophenyl, 4-chlorophenyl, 3,5-dimethoxyphenyl, 4-*tert*-butylphenyl, phenyl,4-trifluoromethylphenyl, $-CH_2CH_2CH_2$-phenyl, 6-quinolyl-C(=O)-, 2-quinoxaline-C(=O)-, 5-chloro-2-benimidazolyl, fluorenylmethoxycarbonyl, 4-chlorobenzyl, 4-methylbenzyl, 3-quinoxalinyl, 3,4-difluorophenyl, or 4-fluorophenyl;
R^6 is a C_3-C_5 branched or unbranched alkyl group, preferably isobutyl or sec-butyl;
R^7 is a natural or unnatural amino acid, preferably N-methylglycine, $-NHCH_2CH_2NHC(=O)-$, L-arginine, D-arginine, L-ornithine, D-ornithine, histidine, citrulline, proline, hydroxyproline, 3-pyridinylalanine, L-N-methylalanine, D-N-methylalanine, aminobutyric acid, or thiazolidine;
R^8 is L-isoleucine-NH_2, D-isoleucine-NH_2, $-CH_2$-cyclopentyl, $-CH_2$-2-tetrahydrofuranyl, tert-butylglycine-NH_2, n-butyl, NH-cyclopentyl, $NHCH_2$-2-furanyl, $-NHCH_2$-pyrininyl, $-NHCH_2$-

cyclohexyl, -NH-2-indolizidinyl, D-leucinol, -NH-isobutyl, L-allo-isoleucine-NH_2, 1-hydroxycycloleucinol, 2-(aminomethyl)-1-ethyl-pyrrolidine, or (S)-NH-2-methylbutyl, but if R^7 is -NH-2-indolizidine, then R^8 is absent; and

R^9 is a one carbon spacer that is preferably =CH- or -C(=O)-; such that when R^9 is =CH-, then ═ is a double bond, and when R^9 is -C(=O)- then ═ is a single bond, and when R^1 is -N=CH- and R^9 is =CH-, then the central ring is a disubstituted imidazole.

Representative compounds according to the present invention include those of the structure:



#	R^1	R^2	R^3	R^4	R^5
1	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
2	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
3	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
4	(R)-CH_2CH_2-	H	a single bond	-C(=O)NH-	(E)-PhHC=$CHCH_2NHCH_2CH_2$-
5	(R)-CH_2CH_2-	Me	para-phenyl	-C(=O)NH-	2-naphthyl
6	(R)-CH_2CH_2-	Me	1,3-propyl	-NHC(=O)-	$PhCH_2CH_2CH_2$-
7	(R)-CH_2CH_2-	Me	-CH_2CH_2C(=O)NH-	-S(=$O)_2$NH-	2-naphthyl
8	(R)-CH_2CH_2-	Me	-CH_2CH_2C(=O)NH-	-S(=$O)_2$NH-	(E)-PhHC=CH-
9	(R)-CH_2CH_2-	Me	-CH_2CH_2C(=O)NH-	-C(=O)NH-	6-quinolinyl-C(=O)-
10	(R)-CH_2CH_2-	Me	para-phenyl	-C(=O)NH-	6-quinolinyl-C(=O)-
11	(R)-CH_2CH_2-	Me	para-phenyl	-C(=O)NH-	2-quinoxalinyl-C(=O)-
12	=CH-CH=CH-	H	para-phenyl	-C(=O)NH-	2-naphthyl
13	=CH-CH=CH-	H	para-phenyl	-C(=O)NH-	2-naphthyl
14	=CH-CH=CH-	H	para-phenyl	-C(=O)NH-	5-chloro-2-indolyl
15	=CH-CH=CH-	H	para-phenyl	-C(=O)NH-	5-chloro-2-indolyl
16	(R)-CH_2CH_2-	Me	-CH_2CH_2C(=O)NH-	-C(=O)NH-	(E)-PhHC=CH-
17	=CH-CH=CH-	H	para-phenyl	-C(=O)NH-	2-naphthyl
18	=CH-CH=CH-	H	para-phenyl	-C(=O)NH-	2-naphthyl
19	(R)-CH_2CH_2-	Me	-CH_2CH_2C(=O)NH-	-C(=O)NH-	2-indole
20	imidazole*	H	para-phenyl	-C(=O)NH-	2-naphthyl
21	(R)-CH_2CH_2-	Me	para-phenyl	-C(=O)NH-	2-naphthyl
22	(R)-CH_2CH_2-	Me	-CH_2CH_2C(=O)NH-	-C(=O)NH-	(E)-PhHC=CH-
23	(R)-CH_2CH_2-	Me	para-phenyl	-C(=O)NH-	2-naphthyl
24	(R)-CH_2CH_2-	Me	para-phenyl	-C(=O)NH-	2-naphthyl
25	(R)-CH_2CH_2-	Me	para-phenyl	-C(=O)NH-	6-quinolinyl
26	(R)-CH_2CH_2-	Me	para-phenyl	-C(=O)NH-	3-quinolinyl
27	(R)-CH_2CH_2-	Me	para-phenyl	-C(=O)NH-	2-quinolinyl
28	(R)-CH_2CH_2-	Me	para-phenyl	-C(=O)NH-	2-indole
29	(R)-CH_2CH_2-	Me	para-phenyl	-C(=O)NH-	(E)-PhHC=CH-
30	(R)-CH_2CH_2-	Me	para-phenyl	-C(=O)NH-	4-methylphenyl
31	(R)-CH_2CH_2-	H	para-phenyl	-NHC(=O)-	4-chlorobenzyl
32	(R)-CH_2CH_2-	H	para-phenyl	-NHC(=O)-	4-methylbenzyl

#	R^1	R^2	R^3	R^4	R^5
33	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
34	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
35	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
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37	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
38	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
39	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
40	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
41	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
42	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
43	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
44	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
45	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
46	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
47	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
48	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
49	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
50	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
51	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
52	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
53	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
54	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
55	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	4-quinolinyl
56	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	6-quinolinyl
57	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	3-quinolinyl
58	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
59	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	3-quinoxalinyl
60	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-quinolinyl
61	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	3-quinolinyl
62	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	6-quinolinyl
63	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	3-isoquinolinyl
64	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-quinolinyl
65	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-indolyl
66	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-indolyl
67	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	4-chlorophenyl
68	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	4-trifluoromethylphenyl
69	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	4-fluorophenyl
70	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	4-methylphenyl
71	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	3-methoxyphenyl
72	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	4-cyanophenyl
73	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	3,4-difluorophenyl
74	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	3-chloro-4-fluorophenyl
75	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	3,5-dimethoxyphenyl
76	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-indolyl
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79	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	4-chlorophenyl
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81	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	4-trifluoromethylphenyl
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83	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	4-trifluoromethylphenyl
84	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2,4-dichlorophenyl
85	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	4-chlorophenyl
86	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	3-quinolinyl
87	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	3-quinolinyl
88	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-quinolinyl

#	R^1	R^2	R^3	R^4	R^5
89	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	3-isoquinolinyl
90	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-quinolinyl
91	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-indolyl
92	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	3-isoquinolinyl
93	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-indolyl
94	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
95	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
96	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
97	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
98	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
99	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
100	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
101	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
102	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
103	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
104	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
105	(S)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
106	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	1-naphthyl
107	(R)-CH_2CH_2-	H	ortho-phenyl	-C(=O)NH-	2-naphthyl
108	(R)-CH_2CH_2-	H	meta-phenyl	-C(=O)NH-	1-naphthyl
109	(R)-CH_2CH_2-	H	meta-phenyl	-C(=O)NH-	2-naphthyl
110	(R)-CH_2CH_2-	H	ortho-phenyl	-C(=O)NH-	1-naphthyl
111	(R)-CH_2CH_2-	H	-CH_2CH_2C(=O)NH-	-NHC(=O)-	2-phenylethyl
112	(R)-CH_2CH_2-	H	-(E)-HC=CHC(=O)NH-	-NHC(=O)-	2-phenylethyl
113	(R)-CH_2CH_2-	H	-CH_2CH_2C(=O)NH-	-NHC(=O)-	(E)-Ph-HC=CH-
114	(S)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	1-naphthyl
115	(S)-CH_2CH_2-	H	ortho-phenyl	-C(=O)NH-	2-naphthyl
116	(S)-CH_2CH_2-	H	meta-phenyl	-C(=O)NH-	1-naphthyl
117	(S)-CH_2CH_2-	H	meta-phenyl	-C(=O)NH-	2-naphthyl
118	(S)-CH_2CH_2-	H	-CH_2CH_2C(=O)NH-	-C(=O)NH-	(E)-Ph-HC=CH-
119	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
120	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
121	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
122	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
123	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
124	(R)-CH_2CH_2-	H	-CH_2CH_2C(=O)NH-	-C(=O)NH-	(E)-Ph-HC=CH-
125	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	FMOC
126	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	5-chloro-2-indolyl
127	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	5-chloro-2-indolyl
128	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	5-chloro-2-indolyl
129	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	5-chloro-2-indolyl
130	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	5-chloro-2-indolyl
131	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	5-chloro-2-indolyl
132	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	6-methyl-3-pyridinyl
133	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	6-methyl-3-pyridinyl
134	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	6-quinolinyl
135	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	6-quinolinyl
136	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	3-quinoxalinyl
137	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	3-quinoxalinyl
138	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	5-chloro-2-benzimidazolyl
139	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	5-chloro-2-benzimidazolyl
140	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	5-chloro-2-benzimidazolyl
141	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	3-quinolinyl
142	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	3-isoquinolinyl
143	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-quinolinyl
144	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-indolyl

#	R^1	R^2	R^3	R^4	R^5
145	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	3-quinolinyl
146	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	3-isoquinolinyl
147	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-quinolinyl
148	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-naphthyl
149	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	3-quinolinyl
150	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	3-isoquinolinyl
151	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-quinolinyl
152	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	6-quinolinyl
153	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	3-quinoxalinyl
154	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	2-indolyl
155	(R)-CH_2CH_2-	H	para-phenyl	-C(=O)NH-	5-chloro-2-indolyl
156	(R)-$CH_2CH_2CH_2$-	H	para-phenyl	-C(=O)NH-	2-naphthyl

* when R^1 is imidazole, the central ring contains no carbonyl group.

#	R^6	R^7	R^8
1	(L)(S)secBu	N-MeGly	Ile-NH_2
2	(L)(S)secBu	-$NHCH_2CH_2NHC$(=O)-	CH_2-cyclopentyl
3	(L)(S)secBu	-$NHCH_2CH_2$-NHC(=O)-	n-Bu
4	(L)(S)secBu	N-MeGly	Ile-NH_2
5	(L)(S)secBu	(D)Arg	Ile-NH_2
6	(L)(S)secBu	(D)Arg	Ile-NH_2
7	(L)(S)secBu	(D)Arg	Ile-NH_2
8	(L)(S)secBu	(D)Arg	Ile-NH_2
9	(L)(S)secBu	(D)Arg	Ile-NH_2
10	(L)(S)secBu	(D)Arg	Ile-NH_2
11	(L)(S)secBu	(D)Arg	Ile-NH_2
12	(DL)(S)secBu	(L)Arg	Ile-NH_2
13	(DL)(S)secBu	(D)ornithine	Ile-NH_2
14	(DL)(S)secBu	(D)Arg	Ile-NH_2
15	(DL)(S)secBu	N-MeGly	Ile-NH_2
16	(L)(S)secBu	(D)Arg	Ile-NH_2
17	(DL)(S)secBu	(D)Arg	Ile-NH_2
18	(DL)(S)secBu	N-MeGly	Ile-NH_2
19	(L)(S)secBu	(D)Arg	Ile-NH_2
20	(L)(S)secBu	(L)Arg	Ile-NH_2
21	(L)(S)secBu	(L)Arg	Ile-NH_2
22	(L)(S)secBu	(L)Arg	Ile-NH_2
23	(L)(S)secBu	N-MeGly	Ile-NH_2
24	(L)(S)secBu	Gly	Ile-NH_2
25	(L)(S)secBu	N-MeGly	Ile-NH_2
26	(L)(S)secBu	N-MeGly	Ile-NH_2
27	(L)(S)secBu	N-MeGly	Ile-NH_2
28	(L)(S)secBu	N-MeGly	Ile-NH_2
29	(L)(S)secBu	N-MeGly	Ile-NH_2
30	(L)(S)secBu	N-MeGly	Ile-NH_2
31	(L)(S)secBu	N-MeGly	NH-i-Bu
32	(L)(S)secBu	N-MeGly	NH-i-Bu
33	(D)(S)secBu	(D)Arg	Ile-NH_2
34	(D)isoBu	(L)ornithine	NH-i-Bu
35	(D)isoBu	(D)Arg	Ile-NH_2
36	(D)isoBu	N-MeGly	Ile-NH_2
37	(D)isoBu	His	Ile-NH_2
38	(D)isoBu	citrulline	NH-i-Bu
39	(D)isoBu	(D)Arg	NH-i-Bu
40	(L)(S)secBu	Pro	NH-i-Bu

#	R⁶	R⁷	R⁸
41	(L)(S)secBu	Hyp	NH-i-Bu
42	(L)(S)secBu	3-pyridinyl-Ala	NH-i-Bu
43	(L)(S)secBu	N-MeAla	NH-i-Bu
44	(L)(S)secBu	(D)N-MeAla	NH-i-Bu
45	(L)(S)secBu	aminobutyric acid	NH-i-Bu
46	(L)(S)secBu	thiazolidine	NH-i-Bu
47	(L)(S)secBu	Pro	Ile-NH_2
48	(L)(S)secBu	Arg	(L)-allo-Ile-NH_2
49	(L)(S)secBu	(L)ornithine	(L)-allo-Ile-NH_2
50	(L)(S)secBu	(D)ornithine	Ile-NH_2
51	(L)(S)secBu	(D)Arg	Ile-NH_2
52	(L)(S)secBu	(L)ornithine	(D) Ile-NH_2
53	(L)(S)secBu	Arg	(D) Ile-NH_2
54	(L)(S)secBu	(D)Arg	NH-i-Bu
55	(L)(S)secBu	(D)Arg	Ile-NH_2
56	(L)(S)secBu	(D)Arg	Ile-NH_2
57	(L)(S)secBu	(D)Arg	Ile-NH_2
58	(L)(S)secBu	(D)ornithine	NH-i-Bu
59	(L)(S)secBu	(D)Arg	Ile-NH_2
60	(L)(S)secBu	(D)Arg	Ile-NH_2
61	(L)(S)secBu	(D)Arg	NH-i-Bu
62	(L)(S)secBu	(D)Arg	NH-i-Bu
63	(L)(S)secBu	(D)Arg	NH-i-Bu
64	(L)(S)secBu	(D)Arg	NH-i-Bu
65	(L)(S)secBu	(D)Arg	NH-i-Bu
66	(L)(S)secBu	N-MeGly	NH-i-Bu
67	(L)(S)secBu	N-MeGly	Ile-NH_2
68	(L)(S)secBu	N-MeGly	Ile-NH_2
69	(L)(S)secBu	N-MeGly	Ile-NH_2
70	(L)(S)secBu	N-MeGly	Ile-NH_2
71	(L)(S)secBu	N-MeGly	Ile-NH_2
72	(L)(S)secBu	N-MeGly	Ile-NH_2
73	(L)(S)secBu	N-MeGly	Ile-NH_2
74	(L)(S)secBu	N-MeGly	Ile-NH_2
75	(L)(S)secBu	N-MeGly	Ile-NH_2
76	(L)(S)secBu	(D)Arg	Ile-NH_2
77	(L)(S)secBu	N-MeGly	Ile-NH_2
78	(L)(S)secBu	N-MeGly	Ile-NH_2
79	(L)(S)secBu	(D)ornithine	NH-i-Bu
80	(L)(S)secBu	(D)Arg	NH-i-Bu
81	(L)(S)secBu	(D)Arg	NH-i-Bu
82	(L)(S)secBu	Pro	NH-i-Bu
83	(L)(S)secBu	Pro	NH-i-Bu
84	(L)(S)secBu	Pro	NH-i-Bu
85	(L)(S)secBu	N-MeGly	NH-i-Bu
86	(L)(S)secBu	N-MeGly	Ile-NH_2
87	(L)(S)secBu	(D)ornithine	Ile-NH_2
88	(L)(S)secBu	(D)ornithine	Ile-NH_2
89	(L)(S)secBu	(D)ornithine	Ile-NH_2
90	(L)(S)secBu	N-MeGly	Ile-NH_2
91	(L)(S)secBu	(D)ornithine	Ile-NH_2
92	(L)(S)secBu	N-MeGly	Ile-NH_2
93	(L)(S)secBu	N-MeGly	Ile-NH_2
94	(L)(S)secBu	Arg	NH-i-Bu
95	(L)(S)secBu	NH-2-indolizidine	[n/a]
96	(L)(S)secBu	N-MeGly	-CH_2-2-THF

#	R^6	R^7	R^8
97	(L)(S)secBu	N-MeGly	(D)-t-BuGly-NH_2
98	(L)(S)secBu	N-MeGly	(DL)-t-BuGly-NH_2
99	(L)(S)secBu	N-MeGly	-NH-cycloleucinol
100	(L)(S)secBu	N-MeGly	-NH-2-($NHCH_2$)-1-Et-pyrrolidine
101	(L)(S)secBu	N-MeGly	-$NHCH_2$-2-furan
102	(L)(S)secBu	N-MeGly	(D)-leucinol
103	(L)(S)secBu	N-MeGly	-$NHCH_2$-2-pyridinyl
104	(L)(S)secBu	Arg	Ile-NH_2
105	(L)(S)secBu	Arg	Ile-NH_2
106	(L)(S)secBu	Arg	Ile-NH_2
107	(L)(S)secBu	Arg	Ile-NH_2
108	(L)(S)secBu	Arg	Ile-NH_2
109	(L)(S)secBu	Arg	Ile-NH_2
110	(L)(S)secBu	Arg	Ile-NH_2
111	(L)(S)secBu	Arg	Ile-NH_2
112	(L)(S)secBu	Arg	Ile-NH_2
113	(L)(S)secBu	Arg	Ile-NH_2
114	(L)(S)secBu	Arg	Ile-NH_2
115	(L)(S)secBu	Arg	Ile-NH_2
116	(L)(S)secBu	Arg	Ile-NH_2
117	(L)(S)secBu	Arg	Ile-NH_2
118	(L)(S)secBu	Arg	Ile-NH_2
119	(L)(S)secBu	Arg	(S)-NH-2-methylbutyl
120	(L)(S)secBu	Gly	(S)-NH-2-methylbutyl
121	(L)(S)secBu	Arg	-NH-CH_2-cyclohexyl
122	(L)(S)secBu	Gly	-NH-CH_2-cyclohexyl
123	(L)(S)secBu	(L)Arg	Ile-NH_2
124	(L)(S)secBu	(L)Arg	Ile-NH_2
125	(L)(S)secBu	(D)Arg	Ile-NH_2
126	(L)(S)secBu	(D)Arg	Ile-NH_2
127	(L)(S)secBu	N-MeGly	Ile-NH_2
128	(L)(S)secBu	(D)ornithine	Ile-NH_2
129	(L)(S)secBu	(D)ornithine	NH-i-Bu
130	(L)(S)secBu	(D)Arg	NH-i-Bu
131	(L)(S)secBu	N-MeGly	NH-i-Bu
132	(L)(S)secBu	(D)Arg	Ile-NH_2
133	(L)(S)secBu	(D)ornithine	Ile-NH_2
134	(L)(S)secBu	N-MeGly	Ile-NH_2
135	(L)(S)secBu	(D)ornithine	Ile-NH_2
136	(L)(S)secBu	N-MeGly	Ile-NH_2
137	(L)(S)secBu	(D)ornithine	Ile-NH_2
138	(L)(S)secBu	(D)Arg	Ile-NH_2
139	(L)(S)secBu	N-MeGly	Ile-NH_2
140	(L)(S)secBu	(D)ornithine	Ile-NH_2
141	(L)(S)secBu	(D)ornithine	-NH-i-Bu
142	(L)(S)secBu	(D)ornithine	-NH-i-Bu
143	(L)(S)secBu	(D)ornithine	-NH-i-Bu
144	(L)(S)secBu	(D)ornithine	-NH-i-Bu
145	(L)(S)secBu	N-MeGly	-NH-i-Bu
146	(L)(S)secBu	N-MeGly	-NH-i-Bu
147	(L)(S)secBu	N-MeGly	-NH-i-Bu
148	(L)(S)secBu	(D)Arg	-$NHCH_2$-cyclohexyl
149	(L)(S)secBu	(D)Arg	-$NHCH_2$-cyclohexyl
150	(L)(S)secBu	(D)Arg	-$NHCH_2$-cyclohexyl
151	(L)(S)secBu	(D)Arg	-$NHCH_2$-cyclohexyl
152	(L)(S)secBu	(D)Arg	-$NHCH_2$-cyclohexyl

#	R^6	R^7	R^8
153	(L)(S)secBu	(D)Arg	-NHCH$_2$-cyclohexyl
154	(L)(S)secBu	(D)Arg	-NHCH$_2$-cyclohexyl
155	(L)(S)secBu	(D)Arg	-NHCH$_2$-cyclohexyl
156	(L)(S)secBu	Arg	-NH-isobutyl

The above representative compounds were synthesized according to the Assembly Procedures below, using the resins shown and purification methods shown.

	Ki(nM)	Assembly Procedure	Resin	Purification Method	MS	MS Method	HPLC RT (min)	HPLC Method
1	2.17	A	Ramage	ppt	800.3	ES+	17.25	a
2	490	B	PAC-PEG	HPLC	769.3	ES+	13.09	a
3	986	B	PAC-PEG	HPLC	743.3	ES+	12.51	a
4	155	A	Ramage	ppt	757.4	ES+	7.15	b
5	0.69	A	Ramage	SPE	899.5	ES+	15.66	a
6	14	A	Ramage	ppt	857.5	ES+	11.14	c
7	328	A	Ramage	SPE	930.3	ES+	10.83	c
8	262	A	Ramage	SPE	906.4	ES+	10.42	c
9	451	A	Ramage	ppt	893.4	ES+	7.55	c
10	38.6	A	Ramage	ppt	900.5	ES+	9.06	c
11	19	A	Ramage	ppt	901.5	ES+	11.49	c
12	2	A	Ramage	SPE	12.582	ES+	895.6	c
13	87	A	Ramage	SPE	853.6	ES+	12.4	c
14	936	A	Ramage	SPE	918.4	ES+	12.81	c
15	410	A	Ramage	SPE	833.4	ES+	13.48	c
16	35	A	Ramage	SPE	870.6	ES+	9.75	c
17	104	A	Ramage	SPE	895.5	ES+	12.6	c
18	165	A	Ramage	SPE	810.3	ES+	13.24	c
19	89	A	Ramage	SPE	883.6	ES+	10.00	c
20	161	A	Ramage	SPE	868.6	ES+	11.28	c
21	0.16	A	Ramage	ppt	890	ES+	11.4	d
22	1.8	A	Ramage	SPE	870	ES+	7.5	d
23	4.6	A	Ramage	SPE	814	ES+	12.3	d
24	2.4	A	Ramage	SPE	800	ES+	17.6	d
25	26.5	A	Ramage	ppt	815	ES+	7.46	d
26	6.9	A	Ramage	ppt	815	ES+	8.4	d
27	22	A	Ramage	ppt	815	ES+	12.6	d
28	23	A	Ramage	ppt	803	ES+	11.6	d
29	14	A	Ramage	ppt	790	ES+	11.6	d
30	27	A	Ramage	ppt	832	ES+	12.7	d
31	233	C	Merrifield	HPLC	741	ES	27.10	e
32	683	C	Merrifield	HPLC	722	ES	26.32	e
33	9.9	A	Rink	SPE	886	FAB+	6.1	f
34	232	C	PS-Aldehyde	SPE	786	FAB+	7.2	f
35	299	A	Rink	SPE	885	FAB+	6.5	f
36	321	A	Rink	SPE	800	FAB+	7.7	f
37	79	A	Rink	SPE	866	FAB+	6.3	f
38	641	C	PS-Aldehyde	SPE	827	FAB+	7.5	f
39	83	C	PS-Aldehyde	SPE	828	FAB+	7.6	f
40	6.0	C	PS-Aldehyde	SPE	769	FAB+	7.6	g
41	8.4	C	PS-Aldehyde	SPE	785	FAB+	6.3	g
42	704	C	PS-Aldehyde	SPE	820	FAB+	6.9	g
43	69	C	PS-Aldehyde	SPE	757	APCI+	8.7	j
44	18	C	PS-Aldehyde	SPE	757	APCI+	8.7	j
45	13	C	PS-Aldehyde	SPE	757	APCI+	8.6	j
46	12	C	PS-Aldehyde	SPE	787	APCI+	8.8	j

	Ki(nM)	Assembly Procedure	Resin	Purification Method	MS	MS Method	HPLC RT (min)	HPLC Method
47	1	A	Rink	SPE	827	APCI+	7.6	j
48	3.5	A	Rink	SPE	885.4	MALDI	6.14	f
49	4.9	A	Rink	SPE	843.4	MALDI	5.94	f
50	1	A	Rink	SPE	843.4	MALDI	6.67	f
51	1.65	A	Rink	SPE	885.4	MALDI	5.09	f
52	0.68	A	Rink	SPE	843.4	MALDI	5.77	f
53	0.41	A	Rink	HPLC	886.4	MALDI	6.06	f
54	51	C	PS-Aldehyde	SPE	827.9	ES+	7.92	f
55	4	A	Rink	SPE	887	ES+	2.70	f
56	9	A	Rink	SPE	887	ES+	2.18	f
57	1	A	Rink	SPE	887	ES+	5.78	f
58	23	C	PS-Aldehyde	SPE	786	ES+	7.61	f
59	10	A	Rink	SPE	887.5	ES+	5.00	f
60	12	A	Rink	SPE	886.6	ES+	6.83	f
61	183	C	PS-Aldehyde	SPE	829	ES+	3.08	g
62	908	C	PS-Aldehyde	SPE	829	ES+	2.47	g
63	550	C	PS-Aldehyde	SPE	829	ES+	6.21	g
64	621	C	PS-Aldehyde	SPE	829	ES+	6.82	g
65	4	C	PS-Aldehyde	HPLC	817	ES+	6.03	g
66	435	C	PS-Aldehyde	SPE	732.3	ES+	7.77	g
67	29	A	Rink	ppt	784.3	API+	6.12	g
68	31	A	Rink	ppt	818.3	API+	7.01	g
69	465	A	Rink	ppt	768.4	API+	5.06	g
70	131	A	Rink	ppt	764.4	API+	5.49	g
71	390	A	Rink	ppt	780.4	API+	4.94	g
72	212	A	Rink	ppt	775.4	API+	4.52	g
73	793	A	Rink	ppt	786.3	API+	5.73	g
74	868	A	Rink	ppt	802.3	API+	6.27	g
75	478	A	Rink	ppt	810.4	API+	5.25	g
76	4.1	A	Rink	SPE	874.5	API+	5.22	g
77	8	A	Rink	ppt	780.4	API+	5.28	g
78	6.7	A	Rink	ppt	818.2	API+	7.39	g
79	297	C	PS-Aldehyde	HPLC	770.3	ES+	5.99	g
80	649	C	PS-Aldehyde	HPLC	812.3	API+	6.25	g
81	740	C	PS-Aldehyde	HPLC	846.5	API+	7.03	g
82	180	C	PS-Aldehyde	ppt	753.3	API+	5.29	h
83	255	C	PS-Aldehyde	ppt	787.3	API+	5.85	h
84	732	C	PS-Aldehyde	ppt	787.2	API+	6.36	h
85	898	C	PS-Aldehyde	SPE	727.3	API+	5.28	h
86	16.0	A	Rink	SPE	801.3	ES+	6.09	i
87	2.1	A	Rink	ppt	844.5	API+	5.79	i
88	5.4	A	Rink	ppt	844.6	API+	7.60	i
89	3.0	A	Rink	ppt	844.5	API+	7.27	i
90	8.9	A	Rink	HPLC	801.3	API+	8.04	i
91	3.1	A	Rink	ppt	832.5	API+	7.12	i
92	7.0	A	Rink	HPLC	801.3	API+	7.66	i
93	12.7	A	Rink	HPLC	789.4	API+	7.55	j
94	0.65	B2	PAC-PEG	HPLC	Mna+=850	API+	25.44	m
95	947	B2	PAC-PEG	HPLC	MH+=734	API+	23.89	l
96	19	B2	PAC-PEG	HPLC	MNa+=793	API+	25.52	m
97	674	B3	Pepsyn KA (100)	HPLC	MH+=801	LC-ES	21.72	l
98	7.8	B3	Pepsyn KAM (175)	HPLC	MH+=800	LC-ES	21.93	l
99	12	B2	PAC-PEG	HPLC	MH+=785	LC-ES	21.99	l
100	605	B2	PAC-PEG	HPLC	MH+=798.9	LC-ES	22.14	l
101	779	B2	PAC-PEG	HPLC	MH+=767.5	LC-ES	22.87	l

	Ki(nM)	Assembly Procedure	Resin	Purification Method	MS	MS Method	HPLC RT (min)	HPLC Method
102	24	B2	PAC-PEG	HPLC	MH+=787.5	LC-ES	21.92	l
103	779	B2	PAC-PEG	HPLC	MH+=778.5	LC-ES	21.76	l
104	0.01	A	Rink	ppt	885.3	FAB+	8.75	n
105	0.41	A	Rink	ppt	885.7	FAB+	8.37	n
106	0.70	A	Rink	ppt	885.5	FAB+	11.77	n
107	213	A	Rink	ppt	885.5	FAB+	12.40	n
108	8.4	A	Rink	ppt	885.6	FAB+	11.85	n
109	0.15	A	Rink	ppt	885.6	FAB+	12.62	n
110	248	A	Rink	ppt	885.5	FAB+	12.40	n
111	2.4	A	Rink	ppt	885.6	FAB+	11.85	n
112	1.2	A	Rink	ppt	885.6	FAB+	12.62	n
113	0.36	A	Rink	ppt	856.6	FAB+	7.31	n
114	73	A	Rink	ppt	885.6	FAB+	11.31	n
115	45	A	Rink	ppt	885.6	FAB+	12.82	n
116	178	A	Rink	ppt	885.6	FAB+	11.45	n
117	174	A	Rink	ppt	885.6	FAB+	12.17	n
118	326	A	Rink	ppt	856.5	FAB+	7.91	n
119	0.11	C	PS-Aldehyde	ppt	842.6	FAB+	14.14	n
120	46	C	PS-Aldehyde	ppt	743.3	FAB+	15.22	n
121	0.5	C	PS-Aldehyde	ppt	868.6	FAB+	15.22	n
122	310	C	PS-Aldehyde	ppt	769.4	FAB+	16.44	n
123	0.36	A	Rink	ppt	885.6	FAB+	12.60	n
124	39	A	Rink	ppt	856.7	FAB+	7.68	n
125	185	A	Rink	SPE	953.4	ES+	7.55	o
126	1.44	A	Rink	HPLC	908.4	ES+	6.30	o
127	6.62	A	Rink	SPE	823.3	ES+	6.57	o
128	1.99	A	Rink	HPLC	866.3	ES+	6	o
129	101	C	PS-Aldehyde	SPE	809.3	ES+	5.65	o
130	85.2	C	PS-Aldehyde	SPE	851.3	ES+	6.73	o
131	87.4	C	PS-Aldehyde	SPE	766.3	ES+	7.05	o
132	70	A	Rink	HPLC	850.5	ES+	2.72	o
133	459	A	Rink	HPLC	808.4	ES+	3.07	o
134	37	A	Rink	SPE	801.4	ES+	3.41	o
135	8	A	Rink	HPLC	844.5	ES+	2.76	o
136	63	A	Rink	SPE	802.3	ES+	5.41	o
137	11	A	Rink	HPLC	844.5	ES+	4.99	o
138	14	A	Rink	SPE	909.5	ES+	6	o
139	65	A	Rink	SPE	824.4	ES+	6	o
140	14	A	Rink	SPE	867.5	ES+	6	o
141	416	C	PS-Aldehyde	SPE	784.3	ES+	4.06	o
142	460	C	PS-Aldehyde	SPE	787.3	ES+	5.88	o
143	822	C	PS-Aldehyde	SPE	787.3	ES+	6.34	o
144	838	C	PS-Aldehyde	SPE	775.3	ES+	5.75	o
145	177	C	PS-Aldehyde	SPE	744.4	ES+	4.41	o
146	317	C	PS-Aldehyde	SPE	744.3	ES+	6.36	o
147	648	C	PS-Aldehyde	SPE	744.4	ES+	6.82	o
148	52	C	PS-Aldehyde	SPE	868.6	ES+	7.24	o
149	296	C	PS-Aldehyde	SPE	869.6	ES+	5.19	o
150	280	C	PS-Aldehyde	SPE	869.6	ES+	7.02	o
151	730	C	PS-Aldehyde	SPE	869.6	ES+	7.38	o
152	663	C	PS-Aldehyde	SPE	869.6	ES+	4.07	o
153	915	C	PS-Aldehyde	SPE	870.6	ES+	6.58	o
154	857	C	PS-Aldehyde	HPLC	857.6	ES+	6.75	o
155	429	C	PS-Aldehyde	HPLC	891.6	ES+	7	o
156	16	C	PS-Aldehyde	HPLC	842.5	ES+	6.50	o

***In Vivo* Profiles**

Oral dose-response activity of several compounds, including I and IV, has been demonstrated in an Acute Hypoxia Model (AHM, Figure 1). A detailed description of this model can be found in WILLIAM L. RUMSEY ET AL., OXYGEN TRANSPORT TO TISSUE XIX (Harrison and Delpy eds., Plenum Press 1997), herein incorporated by reference. Studies with **I** show that orally-dosed ANPCR blockers are capable of diminishing the rise in hypoxia-induced pulmonary pressure at doses as low as 10 mg/kg. This compound caused marked effects on the pulmonary side of the circulation; little if any systemic effect on mean arterial pressure (MAP) was observed.

Similar results were observed upon oral administration of peptide **IV**. Peptide **IV** decreased the rise in hypoxia-induced pulmonary pressure at 30 mg/kg without significant systemic effects.

Radioimmunoassay

Studies were performed (Figure 2) to determine whether oral administration of **I** would increase plasma levels of ANP concomitant with changes in right ventricular systolic pressure in the acute hypoxia model *in vivo*. Compared with vehicle controls, rats exposed to **I** showed a 1.7-fold increase in immunoreactive plasma concentrations of ANP (mean±SE: 19.91±1.24 vs. 33.85±4.54 pg/ml, respectively; Figure 2, top panel). These values were statistically different at the $P<0.05$ level. Similar results were observed with **IV** (Figure 2, bottom panel).

Administration and Use

Compounds of the present invention are shown to have natriuretic, diuretic and hypotensive activity in the intact mammal, and may possess vasorelaxant activity or inhibit the release of aldosterone and renin. Thus, these compounds, and compositions containing them, may be used as therapeutic agents in the treatment of various edematous states such as, for example, congestive heart failure, nephritic syndrome and hepatic cirrhosis, pulmonary disease, in addition to hypertension and renal failure due to ineffective renal perfusion or reduced glomerular filtration rate.

The present invention also provides compositions comprising an effective amount of compounds of the present invention, including the nontoxic addition salts, amides and esters

thereof, which may, serve to provide the above-recited therapeutic benefits. Such compositions can also be provided together with physiologically-tolerable liquid, gel or solid diluents, adjuvants and excipients. The compounds of the present invention may also be combined with other compounds known to be adjuvants for, or otherwise used as, therapeutic agents for the above or related indications.

These compounds and compositions may be administered to humans in a manner similar to other therapeutic agents and, additionally, to other mammals for veterinary use, such as with domestic animals. In general, the dosage required for therapeutic efficacy will range from about 0.01 to 1000 mg/kg, more usually 0.1 to 100 mg/kg of the host body weight. Alternatively, dosages within these ranges can be administered by constant infusion over an extended period of time until the desired therapeutic benefits have been obtained.

Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also be emulsified. The active ingredient is often mixed with diluents or excipients which are physiologically tolerable and compatible with the active ingredient. Suitable diluents and excipients are, for example, water, saline, dextrose, glycerol, or the like, and combinations thereof. In addition, if desired, the compositions may contain minor amounts of auxiliary substances such as wetting or emulsifying agents, stabilizing or pH-buffering agents, and the like.

The compositions are conventionally administered parenterally, by injection, for example, either subcutaneously or intravenously. Additional formulations which are suitable for other modes of administration include suppositories, intranasal aerosols, and, in some cases, oral formulations. For suppositories, traditional binders and excipients may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10% preferably 1%-2%. Oral formulations include such normally-employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, cellulose, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained-release formulations, or powders, and contain 10%-95% of active ingredient, preferably 25%-70%.

The peptide compounds may be formulated into compositions as neutral or salt forms. Pharmaceutically-acceptable nontoxic salts include the acid addition salts (formed with the

free amino groups) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or organic acids such as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups may be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

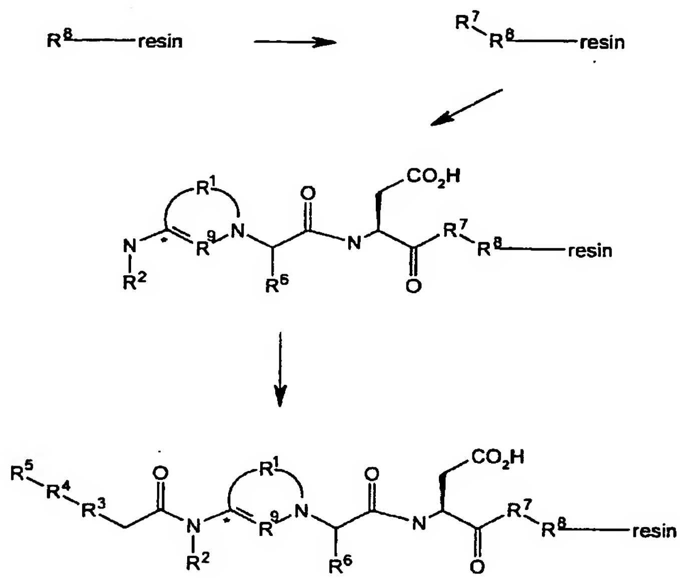
In addition to the compounds of the present invention which display natriuretic, diuretic or vasorelaxant activity, compounds of the present invention may also be employed as intermediates in the synthesis of such useful compounds. Alternatively, by appropriate selection, compounds of the present invention whose activity levels are reduced or eliminated entirely can serve to modulate the activity of other diuretic, natriuretic or vasorelaxant compounds, including compounds outside the scope of the present invention, by, for example, binding to alternate receptors, stimulating receptor turnover, or providing alternate substrates for degradative enzyme or receptor activity and thus inhibiting these enzymes or receptors. When employed in this manner, such compounds may be delivered as admixtures with other active compounds or may be delivered separately, for example, in their own carriers.

Compounds of the present invention may also be used for preparing antisera for use in immunoassays employing labeled reagents, usually antibodies. Conveniently, the polypeptides can be conjugated to an antigenicity-conferring carrier, if necessary, by means of dialdehydes, carbodiimide or using commercially-available linkers. These compounds and immunologic reagents may be labeled with a variety of labels such as chromophores; fluorophores such as, e.g., fluorescein or rhodamine; radioisotopes such as ^{125}I, ^{35}S, ^{14}C, or ^{3}H; or magnetized particles, by means well known in the art.

These labeled compounds and reagents, or labeled reagents capable of recognizing and specifically binding to them, can find use as, e.g., diagnostic reagents. Samples derived from biological specimens may be assayed for the presence or amount of substances having a common antigenic determinant with compounds of the present invention. In addition, monoclonal antibodies may be prepared by methods known in the art, which antibodies can find therapeutic use, e.g., to neutralize overproduction of immunologically-related compounds *in vivo*.

Synthesis

Compounds within the scope of the present invention may be synthesized chemically by means well known in the art. One example of such a scheme may be generally depicted as:



where the starting material is attached to a resin and the compound is constructed by the successive addition of various building blocks. Alternatively, the resin may be attached to a starting material that will end up in a more central location of the desired compound; and through the use of commonly-known protecting groups, the compound may be extended in multiple directions.

Examples

Purification methods:

SPE = solid phase extraction;

ppt = precipitation from ether;

HPLC = preparative HPLC.

HPLC methods:

(a) 20% to 80% ACN in 25 min. Monitored at 254 nm;

(b) 20% to 80% ACN in 25 min. Monitored at 210 nm;

(c) 10% to 60% ACN in 10 min. Monitored at 210 nm and 254 nm;

(d) 20-80% ACN/H_2O (both w/ 0.1% TFA) over 20 min hold 80% ACN for 5 min.;

(e) 10-50% ACN/H_2O/0.1%TFA over 30 min on a Dynamax C_{18}, 60 Å, 4.6 mm x 300 mm column at 210 nm and 254nm;

(f) 4.6 mm x 25 cm Vydac C_{18} Peptide/Protein Column (5 mm) 30-70% CH_3CN/H_2O (+0.1% CF_3CO_2H) over 20 min. 1.5 mL/min, T=35 °C; l = 220 nm;

(g) 4.6 mm x 25 cm Vydac C_{18} Peptide/Protein Column (5 mm) 30-60% CH_3CN/H_2O (+0.1% CF_3CO_2H) over 10 min. 1.5 mL/min, T=35 °C; l = 220 nm;

(h) 4.6 mm x 5 cm Varian Microsorb Column (3 mm) 30-60% CH_3CN/H_2O (+0.1% CF_3CO_2H) over 8 min. 1.0 mL/min, T=35 °C; l = 220 nm;

(i) 4.6 mm x 5 cm Varian Microsorb Column (3 mm) 5-80% CH_3CN/H_2O (+0.1% CF_3CO_2H) over 12 min. 1.0 mL/min, T=35 °C; l = 220 nm;

(j) 4.6 mm x 5 cm Varian Microsorb Column (3 mm) 10-90% CH_3CN/H_2O (+0.1% CF_3CO_2H) over 10 min. 1.0 mL/min, T=35 °C; l = 220 nm;

(k) Preparative Method: Using a Waters LC 4000 HPLC system with Waters 991 PDA detector. Column: Dynamax 25 mm id. x 20cm 300 Å column No. C_{18}-83-223-C with Guard column using a water + 0.1% [v/v] trifluoroacetic acid /acetonitrile + 0.1% [v/v]trifluoroacetic acid gradient at a flow rate of 12 mL/min. l=220 nm;

(l) Using a Waters LC 4000 HPLC system with Waters 991 PDA detector. Column: YMC 4.6 mm x 250mm ODS-A S-5 C_{18} 120 Å column spherical particle-5m using a 20 to 70% water + 0.1% [v/v] trifluoroacetic acid /acetonitrile + 0.1% [v/v]trifluoroacetic acid gradient over 20 min. at a flow rate of 1.4ml/min. l=220 nm;

(m) Using a Waters LC 600E HPLC system with Waters tunable-absorbance UV detector. Column: Vydac 218TP54 4.6 mm x 250mm C_{18} with guard column 300 Å column 5 μm particle size using a 10 to 50% water + 0.1% [v/v] trifluoroacetic acid /ACN + 0.1% [v/v]trifluoroacetic acid gradient over 30 min. at a flow rate of 1.5ml/min. l=220 nm;

(n) Dynamax C_{18} column, 25 cm x 4.6 mm, 60A, 8 μm, 1.5 mL/min, 20%-60% ACN/ H_2O (0.1%TFA) over 20 min, 215 nm and 254 nm;

(o) Dynamax C_{18} column, 5 cm x 4.6 mm, 100 Å, 3 μm, 1 mL/min, 20%-60% ACN/H_2O (0.1%TFA) over 7.5 min, 215 nm and 254 nm.

Central Ring Intermediate Examples:

Synthesis of N-fluorenylmethyloxycarbonyl-N-Me-D-freidingerlactam-L-isoleucine.

Loading of N-fluorenylmethyloxycarbonylfreidingerlactam-L-isoleuicine to 2-chlorotritylchloride resin, 1% DVB. The 2-chlorotritylchloride resin (25g) was swelled in CH_2Cl_2 (300 mL) and drained. DIPEA (12.5 mL) was dissolved in 175 mL dry CH_2Cl_2 and added to the swelled resin. The N-fluorenylmethyloxycarbonylfreidingerlactam-L-isoleucine (11.82g) was dissolved in 175 mL of dry CH_2Cl_2, followed by 12.5 mL DIPEA with vigorous stirring. This was added to the resin and reaction was shaken on a mechanical shaker for three hours. The resin was filtered, washed 500 mL 17:2:1 CH_2Cl_2:MeOH:DIPEA, and CH_2Cl_2 (8X). The recovered filtrates were combined washed 2X 1N HCl, stripped to an off white solid, weight recovered starting material 1.98g (17%).

2-Nitrobenzenesulfonamide protection. The resin was swelled in DMF and 20% piperidine/DMF was added (200 mL) and N_2 was bubbled through for 20 minutes. The resin was filtered and the deprotection repeated. Resin was washed 8X DMF, Kaiser test, strong positive. The resin was washed 8X dry THF. A solution of 24 mL DIPEA in 500 mL dry THF was added to the resin, followed by portion-wise addition of 20.32 g 2-nitrobenzenesulfonyl chloride dissolved/diluted to 92 mL with CH_2Cl_2. The resin was shaken for 4 hrs when the cocktail was filtered and resin washed 8X THF, Kaiser test negative.

Mitsunobu. To the THF-swelled resin was added 30.05 g triphenylphosphine dissolved/diluted to 57 mL in dry THF, followed by a solution of 9.3 mL dry MeOH in 375 mL dry THF. Diethylazodicarboxylate (DEAD) (18.0 mL) was dissolved/diluted to 114 mL with dry THF and added to the resin. After shaking the reaction for 1.5 hrs the cocktail was filtered and the resin washed 8X THF, 8X CH_2Cl_2, 8X Et_2O, dried over N_2 and stored under refrigeration over night.

Sulfonamide cleavage. The resin was swelled in DMF and 250 mL of a 1 M (66.1 g diluted to 500 mL with DMF) solution of benzenethiol sodium salt in DMF was added and shaken for 1 hr. The resin was drained, washed 8X DMF and the remaining 250 mL of the 1 M solution was added and shaken for an additional hour. The resin was filtered and washed 3X DMF, 3X MeOH, 3X DMF, 3X MeOH, 8X CH_2Cl_2, Npit test positive.

Fmoc protection. To the CH_2Cl_2 swelled resin was added a solution of 12 mL DIPEA in 150 mL dry CH_2Cl_2. Fmoc-Cl (23.73 g) was dissolved in 150 mL dry CH_2Cl_2 followed by

12 mL DIPEA. This was added to the resin and reaction shaken for three hours. The resin was filtered and washed 8X CH_2Cl_2, Npit Test, negative.

Cleavage of N-fluorenylmethyloxycarbonyl-N-Me-D-freidingerlactam-L-isoleucine from the resin. A 1% TFA/CH_2Cl_2 solution (250 mL) was added to the resin and shaken for 20 minutes and drained into a round-bottom flask, this was repeated again with 250 mL fresh 1% TFA/ CH_2Cl_2 for 25 minutes and collected. The organics were striped, leaving a light brown solid which was placed under vacuum overnight. Weight of material 10.80 g (quantitative yields, based on loaded starting material) APCI-MS: M+1, 451 (20%); M-18, 433 (50%); M-46, 405 (50%); M-222, 229 (100%) ^{1}H NMR (d_6-DMSO/TFA shake, 300 MHz) δ 0.839 (m, 3H, CH_3) δ 0.941 (m, 3H, CH_3) δ 1.033-1.127 (m, 1H, CH_2) δ 1.374 (m, 1H, CH) δ 1.985 (m, 2H, CH_2) δ 2.219 (m, 1H, CH_2) δ 2.702 (s, 3H, CH_3) δ 3.345-3.430 (m, 2H, CH_2) δ 4.263-4.424 (m, 4H, 2CH, CH_2) δ 4.680-4.794 (m, 1H, CH) δ 7.354 (t, 2H, CH, J=7.2) δ 7.434 (t, 2H, CH, J= 7.4) δ 7.668 (d, 2H, CH, J=7.2) δ 7.893 (d, 2H, CH, J= 7.5)

Synthesis of N-Fluorenylmethyloxycarbonyl-D-freidingerlactam-L-isoleucine

t-Boc-D-methionine-L-isoleucine methyl ester. Boc-D-methionine (24.2 g, 97.2 mmol), L-isoleucine methyl ester hydrochloride (17.7 g, 97.2 mmol), hydroxybenztriazole hydrate (16.3 g, 117 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (22.5 g, 117 mmol) and diisoproplylethylamine (35ml, 200 mmol) were dissolved in DMF (300 mL). The reaction was stirred under nitrogen for 16 hours, then it was diluted with water (1000 mL) and extracted with ethyl acetate (2 x 250 mL). The combined organics were washed with 1M HCl (100 mL), water (2 x 100 mL), saturated aqueous sodium bicarbonate (100 mL) and brine (100 mL). The organics were dried with magnesium sulfate and concentrated by rotary evaporation. The resulting oil was diluted with ether (25 mL) and seeded with crystals. The product was allowed to crystallize for 30 minutes then collected by vacuum filtration, then washed with a 1 to 5 ether/hexanes mixture (100 mL). A second crop of crystalline product was recovered from the mother liquors. It was washed with a 1 to 10 ether/hexanes mixture. The second crop was determined by HPLC to be of sufficient quality to combine with the first. The combined product was dried *in vacuo* for 30 minutes at 50 °C to yield 25.3 g white solid (89%).

Boc-D-freidingerlactam-L-isoleucene methyl ester. t-Boc-D-methionine-L-isoleucine methyl ester (25.3 g, 67.2 mmol) was dissolved in dry methylene chloride (250 mL) under nitrogen and chilled in an ice bath. Trimethyloxonium tetrafluoroborate (9.94 g, 67.2 mmol)

was added in one portion. The ice bath was removed and the reaction was allowed to react for 3 hours. HPLC analysis of an aliquot showed there was no remaining starting material. Dry, powdered potassium carbonate (27.9 g, 202 mmol) was added and the reaction was stirred vigorously with an overhead stirrer and refluxed for 16 hours. HPLC analysis of an aliquot showed there was no remaining intermediate. The reaction was diluted with methylene chloride (400 mL) and washed with water (5 x 500 mL) then brine (200 mL). The organics were dried with magnesium sulfate and rotary evaporated to a white solid. The residue was dissolved in refluxing methylene chloride (25 mL), and precipitated by addition of hexanes (300 mL). The solids were collected by vacuum filtration and washed with hexanes (100 mL). A second crop of solids was obtained from the mother liquor and washed with hexanes (50 mL). The second crop was determined by HPLC to be of sufficient quality to combine with the first. The combined product was dried *in vacuo* at 50 °C for 30 minutes to yield 17.0 g white solid (77%).

Boc-D-freidingerlactam-L-isoluecene. Boc-D-freidingerlactam-L-isoleucene methyl ester (15.6 g, 47.5 mmol) was dissolved in THF (60 mL) and methanol (60 mL). Lithium hydroxide (4.2 g, 100 mmol) dissolved in water (60 mL) was added. After one hour, TLC analysis showed no remaining starting material. All the solvent was removed by rotary evaporation. The resulting white solid was dissolved in water (300 mL), washed with methylene chloride (50 mL), and acidified with 1M HCl (105 mL). A white precipitate formed. It was extracted from the aqueous phase with ethyl acetate (700 mL), washed with brine (50 mL), dried with magnesium sulfate, and concentrated to a white solid (11.4 g) by rotary evaporation. HLPC analysis showed 17% epimerization. The single diastereomer was obtained by refluxing the solid in ethyl acetate (125 mL), allowing it to stand at room temperature for one hour, collecting the solid by vacuum filtration and drying *in vacuo* at 50 °C for 30 minutes. The resulting white solid (8.9 g, 60%) was determined to be the pure single diastereomer by HPLC and the correct product by ^{1}H NMR.

N-Fluorenylmethyloxycarbonyl-D-freidingerlactam-L-isoleucine. Boc-D-freidingerlactam-L-isoluecene (8.86 g, 28.2 mmol) was suspended in methylene chloride and trifluoroacetic acid (30 mL) was added. After 1.5 hours the volatiles were removed by rotary evaporation. Methylene chloride (2 x 50 mL) was added and evaporated to rid remaining TFA. The residue was cooled in an ice bath and dioxane (42ml) and 10% aqueous sodium carbonate (71ml) were added. FMOC chloride (8.8 g, 33.9 mmol) was added to the resulting

solution in four portions. After 18 hours the reaction was diluted with water until a clear solution was obtained (400 mL total volume). This was washed with ether (50 mL), and the aqueous phase was acidified with 1M HCl to pH = 3. The aqueous layer was extracted with methylene chloride (4 x 100 mL). The combined methylene chloride extracts were washed with brine (50 mL) and dried with magnesium sulfate. The solvent was removed by rotary evaporation to give a white foam. This was dissolved in n-butyl acetate (300 mL), and crystallized by addition of hexanes (200 mL). The white solid was collected by vacuum filtration and dried *in vacuo* 50 °C for 30 minutes (9.36 g, 76%).

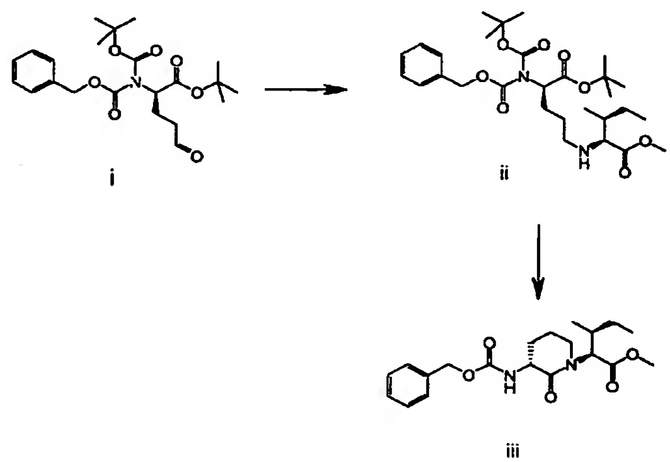
Synthesis of N-fluorenylmethyloxycarbonylpyridone-D,L-isoleucine. A solution of isoleucine *t*-butyl ester hydrochloride (16.7 g) in EtOH (48 mL) was added to a solution of diethyl 3-ethoxyallylidenemalonate (18.0 g) in EtOH (100 mL) followed by diisopropylethylamine (19.4 mL). After 2 h, NaOEt in EtOH (55.5 mL of a 21 wt.% solution) was added. After 2.5 h, H_2O (70 mL) was added to the reaction. The reaction solution was acidified to pH = 5 by the addition of 1N HCl 15 h later. The ethanol was removed from the reaction under reduced pressure and the residue was partitioned between 1N HCl (300 mL) and $CHCl_3$ (300 mL). The organic layer was dried with Na_2SO_4, filtered through celite and concentrated to a viscous oil. The material was purified by silica gel flash chromatography (6 cm x 25 cm) using a gradient from 20 to 100% EtOAc in hexane. The product (a 3-carboxypyridone)(13.9 g) was obtained as an oil (R_f = 0.4 in 30% EtOAc in hexane). Diphenylphosphorylazide (11.2 mL) and triethylamine (7.5 mL) were added to a solution of the substituted 3-carboxypyridone (13.8 g) in dioxane (100 mL). After heating to 100 °C for 1 h, benzyl alcohol (5.2 mL) was added and the reaction mixture was continually heated at 100 °C for 16h. The reaction was cooled to room temperature, the solvent was removed under reduced pressure and the obtained residue was partitioned between EtOAc (300 mL) and 1:1 1N HCl and sat. NaCl solution (200 mL). The organic layer was washed with 1:1 1N HCl and sat. NaCl solution (200 mL). The organic layer was dried with Na_2SO_4, filtered through celite and concentrated. The material was purified by silica gel flash chromatography (5 cm x 30 cm) using a gradient from 10 to 50% EtOAc in hexane. The benzyloxycarbonyl *N*-protected product obtained (15.2 g; R_f = 0.7 in 30% EtOAc in hexane) was dissolved in EtOH (150 mL) and 10% Pd-C (3 g) was added. The reaction mixture was kept under H_2 (45 psi) for 5 h. The reaction solution was filtered through celite and the filtrate was concentrated giving the corresponding 3-aminopyridone (9.9 g). 9-Fluorenylmethyl chloroformate(11.0 g) was added

in small batches over the course of 1 h to a cooled solution (4 °C) of the 3-aminopyridone (9.9 g) in 10% aqueous Na_2CO_3 (89 mL) and dioxane (53 mL). The reaction was allowed to warm to room temperature over 1 h. The solvents were removed under reduced pressure and the residue was partitioned between EtOAc (300 mL) and 1N HCl (200 mL). The organic layer was washed with 1N HCl (100 mL). The combined aqueous washings were extracted with EtOAc (40 mL). The combined organic extracts were dried with Na_2SO_4, filtered through celite and concentrated. The material was loaded on a silica gel flash column (5 x 20 cm) and the product was eluted off using a gradient of 10 to 30% EtOAc in hexane. The FMOC-protected product (15.4 g; R_f = 0.75 in 30% EtOAc in hexane) was obtained as an off-white foam. The FMOC-aminopyridone (15.3 g) was dissolved in 3:1 CH_2Cl_2 in TFA (100 mL). After 14 h the solvents were removed under reduced pressure. The residue was concentrated from Et_2O (3 x 20 mL) to give the product (N-fluorenylmethyloxycarbonyl pyridone-isoleucine) (13.6 g) as a foam.

Synthesis of N-fluorenylmethyloxycarbonylimidazole-L-isoleucine. A solution of L-isoleucine-*O-t*-butyl ester hydrochloride (7.10 g) and $NaHCO_3$ (2.80 g) in MeOH (150 mL) and H_2O (80 mL) were added to 1,4-dinitroimidazole (5.0 g) at 0 °C. After 4.5 h the MeOH was removed under reduced pressure and the remaining solution was partitioned between EtOAc (300 mL) and 1N HCl (200 mL). The organic layer was washed with 1N HCl (200 mL x 2), dried with Na_2SO_4, filtered through celite and concentrated. The residue was purified by silica gel flash chromatography (6 x 23 cm) using a gradient from 20 to 67% EtOAc in hexane to afford the mononitroimidazole product (8.17 g; R_f of 0.33 in 30% EtOAc in hexane). The obtained material and 10% Pd-C (1.4 g) were reacted in EtOH (135 mL) under 47 psi H_2 for 2 h. The reaction mixture was filtered through celite and the filtrate was concentrated under reduced pressure. The amino-imidazole product (7.05 g) was dissolved in 10% Na_2CO_3 (aq) (70 mL) and dioxane (42 mL) and the solution was cooled in an ice-water bath. Then, 9-fluorenylmethyl chloroformate (7.93 g) was added in small portions over the course of 35 min. The ice bath was removed and the reaction was continued for another 3 h. The reaction mixture was partitioned between 250 mL EtOAc and 350 mL 1N HCl. The organic layer was washed with 1N HCl (200 mL x 2), dried with Na_2SO_4, filtered through celite and concentrated. The residue was applied to a silica gel flash column (6 x 23 cm). The product (5.07 g; R_f = 0.5 in 50% EtOAc/hexane) was eluted off the column using a gradient of 20 to 67% EtOAc in hexane. The FMOC-amino compound (5.07 g) was dissolved in 3:1

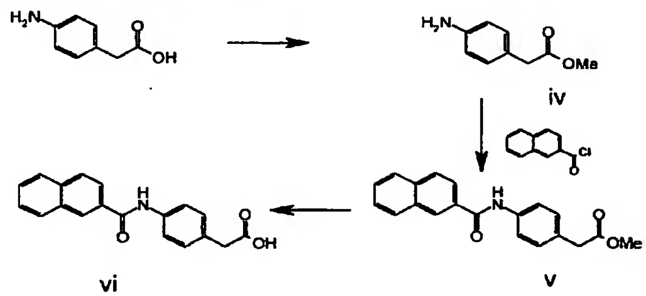
CH_2Cl_2:TFA. After 16 h the solvents were removed under reduced pressure. The viscous oil was concentrated from Et_2O (30 mL x 3) until a foam resulted.

Synthesis of N-benzyloxycarbonyl-D-freidingerlactam-L-isoleucine



Aldehyde i (2.18 g, 5.16 mmol) and L-isoleucine methyl ester hydrochloride (1.09 g, 6.0mmol, 1.2 eq) were dissolved in 30 mL methanol. Sodium cyanoborohydride (0.95 g, 15 mmol) was added and the mixture was stirred for 2 hours, then diluted with 150 mL ethyl acetate and washed with aqueous 10% sodium carbonate, water and brine. The organics were dried with anhydrous magnesium sulfate and the volatiles were removed by rotary evaporation. Column chromatography on silica gel using 25% ethyl acetate in hexanes afforded 1.45 g (51%) of intermediate ii as a colorless oil. A portion (0.75 g, 1.36 mmol) of intermediate ii was dissolved in 10 mL methylene chloride and 10 mL trifluoroacetic acid was added. After 2 hours the volatiles were removed by rotary evaporation, and the residue was dissolved in 25 mL dry THF to which was added DIEA (1.1 mL, 4.5 eq), HOBT (0.42 g, 2.2 eq), and EDC (0.58 g, 2.2 eq). The reaction was stirred under nitrogen atmosphere for 16 hours, then diluted with ethyl acetate and washed with water and brine. Column chromatography on silica gel with a gradient of ethyl acetate in methylene chloride afforded 0.35 g (69%) of intermediate iii. All of intermediate iii was dissolved in 30 mL ethanol, and hydrogenated for 3 hours on a Parr shaker with 50 psi hydrogen and Pearlman's catalyst.

R^5-R^4-R^3-CH_2CO_2H Intermediate Examples

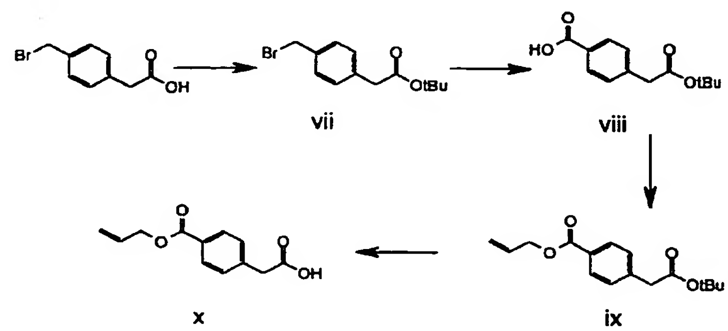


4-(2-Naphthaloylamido)phenylacetic acid

p-Aminophenylacetic acid (5.0 g, 33.07 mmol) was dissolved in MeOH (50 mL) and placed in an ice water bath followed by drop-wise addition of H_2SO_4 (4 mL). After stirring for 10 min, the dark colored reaction mixture was heated to 60 °C for one hour. The reaction was allowed to cool to room temperature and while stirring the mixture was slowly quenched by addition of $NaHCO_3$ (sat. 100 mL). The reaction mixture was washed with ether (3 x 200 mL). The combined organics were washed with water and brine, then dried ($MgSO_4$), and evaporated under vacuum to provide 4.26 g (76%) of the dark crude methyl ester iv that was used directly in the next step. The crude methyl ester (4.26 g, 25.81 mmol) was dissolved in CH_2Cl_2 (50 mL) and Et_3N (3.96 mL) was added. 2-Naphthoyl chloride (4.92 g, 25.81 mmol) dissolved in CH_2Cl_2 (5 mL) was added drop-wise and reaction allowed to stir for 2 hours under N_2. The reaction was quenched with $NaHCO_3$ (100 mL) and washed with CH_2Cl_2 (3 x 150 mL). The organics were combined and washed with brine, dried ($MgSO_4$), and evaporated under vacuum. The material was precipitated with Et_2O, filtered and washed with Et_2O several times and dried in an vacuum oven at 50 °C to provide 6.86 g (83%) of amide v as an off-white solid that was used directly in the next step. The crude off-white solid (6.3 g, 19.7 mmol) was dissolved in THF (253 mL) and MeOH (63 mL). Next, $LiOH.H_20$ (1.66 g) dissolved in water (63 mL) was added drop-wise. The reaction mixture was stirred for 30 min and reaction adjusted to pH 2 with 1N HCl. Reaction mixture was washed with EtOAc (3 x 300 mL). The combined organics were washed with brine, dried ($MgSO_4$), and evaporated under vacuum. The crude material was precipitated with Et_2O, filtered and washed with Et_2O several times and dried in an vacuum oven at 50 °C to provide 5.75 g (95.7%) of a pale white

solid vi that was used for peptide N-capping. [1]H NMR (DMSO-d_6) δ 10.42 (s, 1H), 8.58 (s, 1H), 8.11-8.00 (m, 4H), 7.75 (d, J = 8.4 Hz, 2H), 7.67-7.60 (m, 2H), 7.25 (d, J = 8.4 Hz, 2H), 3.55 (s, 2H).

4-(Allylacetate)phenylacetic acid



4-(Bromomethyl)phenylacetic acid (20.0 g, 87.3 mmol) was stirred in anhydrous CH_2Cl_2 (200 mL) under N_2 and cooled in an ice-water bath. DMF (cat., 0.4 mL, 0.25 eq) was added followed by drop-wise addition of oxalyl chloride (9.1 mL) to the stirring cooled solution and the 4-(bromomethyl)phenylacetic acid began to slowly dissolve. After 2 hours, the solvent and excess oxalyl chloride was removed under vacuum at 50 °C and the crude acid chloride was chased with anhydrous toluene (2x 10 mL). In a separate 3-neck flask with a condenser charged with t-BuOH (500 mL, Aldrich distilled), the t-BuOH was purged with a stream of N_2 while stirring under N_2 for 15 min. The t-BuOH was heated to 55 °C and DIPEA (22.8 mL) was added followed by addition of the crude acid chloride dissolved in CH_2Cl_2 (100 mL). After 30 min, the excess t-BuOH was evaporated under vacuum at 60 °C and the salts were precipitated with Et_2O, filtered, washed with Et_2O, and discarded. The Et_2O mother liquors were combined and concentrated. Silica gel flash chromatography of the crude product (5% ethyl acetate-hexane) provided 17.2 g (69%) of the t-butyl ester vii as a clear and colorless oil. [1]H NMR ($CDCl_3$) δ 7.3 (d, J = 8.3 Hz, 2H), 7.2 (d, J = 8.3 Hz, 2H), 4.5 (s, 2H), 3.5 (s, 2H), 1.3 (s, 9H). Mass spectroscopy did not provide any interpretable peaks or fragments.

t-Butyl ester vii (17.2 g, 60.35 mmol) was dissolved in DMSO (120 mL) and $NaNO_2$ (16.66 g, 4 eq.) was added in one portion followed by addition of CH_3CO_2H (34.55 mL, 10.0 eq.). A condenser was connected to the flask and the stirred mixture was heated to 35 °C

overnight. The reaction was cooled to room temperature and quenched with water (200 mL) and allowed to stir for 15 min. The mixture was washed with Et_2O (3 x 150 mL). The Et_2O layers were combined, washed with brine, then with $NaHCO_3$ (sat.) (2 x 100 mL). The $NaHCO_3$ layers were combined and acidified to pH = 4 with slow addition of concentrated HCl. The acidic aqueous layer was washed with CH_2Cl_2 (3 x 150 mL), the organics were combined and washed with brine, dried ($MgSO_4$), and concentrated to provide <u>viii</u> (8.2 g, 60%) as a white solid. ^1H NMR (DMSO-d_6) δ 7.9 (d, J = 8.4 Hz, 2H), 7.3 (d, J = 8.4 Hz, 2H), 3.6 (s, 2H), 1.4 (s, 9H). Mass spectroscopy did not provide any interpretable peaks or fragmentation's.

The benzoic acid derivative <u>viii</u> (7.57 g, 32.07 mmol) was dissolved in DMF (100 mL) and K_2CO_3 (4.4 g, 32.07 mmol) was added as a solid and the mixture was stirred under N_2 for 15 min followed by addition of allyl bromide (2.9 mL 1.05 eq). After 2 hours, the reaction was added to ethyl acetate and washed with water (150 mL) and brine (5 x 200 mL). The organic layer was dried ($MgSO_4$), and concentrated. Silica gel chromatography (5% ethyl acetate in hexane) of the crude oil provided <u>ix</u> as a colorless and clear oil (7.5 g, 85%). ^1H NMR (DMSO-d_6) δ 7.9 (d, J = 8.4 Hz, 2H), 7.4 (d, J = 8.1 Hz, 2H), 6.0 (m, 1H), 5.4(dd, J = 3 Hz, J = 17.2 Hz, 1H), 5.2 (dd, J = 3 Hz, J = 10.5 Hz, 1H) 4.8 (d, J = 5.4 Hz, 2H), 3.7 (s, 2H), 1.4 (s, 9H). Mass spectroscopy provided fragmentation of 221 indicating lose of the t-butyl group (-57).

Compound <u>ix</u> (7.5 g, 27.17 mmol) was dissolved in CH_2Cl_2 (200 mL) and TFA (63 mL) dissolved in CH_2Cl_2 (100 mL) was added drop-wise to the stirring solution. After 4 hours, the solvent was removed under vacuum. Silica gel flash chromatography of the crude product (5% MeOH/ CH_2Cl_2) provided <u>x</u> as a white solid (5.9 g, 95%). ^1H NMR (DMSO-d_6) δ 7.9 (d, J = 8.4 Hz, 2H), 7.4 (d, J = 8.1 Hz, 2H), 6.0 (m, 1H), 5.4(dd, J = 3 Hz, J = 17 Hz, 1H), 5.2 (dd, J = 3.0, Hz, J = 10.5 Hz, 1H) 4.8 (d, J = 5.1 Hz, 2H), 3.7 (s, 2H). MS 220.3 (M^+), 163.2 (-57)

N-(2-naphthoyl)-3-aminophenylacetic acid and N-(1-naphthoyl)-3-aminophenylacetic acid.

Methyl 3-aminophenylacetate. 3-Aminophenylacetic acid (3.8 g, 25 mmol) was dissolved in methanol (50 mL) and sulfuric acid (2 mL, 36 mmol). After the reaction was stirred for 8 hours the solvents were removed by rotary evaporation. The residue was partitioned between ethyl acetate (100 mL) and 10% sodium carbonate (50 mL). The organic

phase was washed with brine (30 mL) and dried over magnesium sulfate. The solvent was removed by rotary evaporation to afford methyl 3-aminophenylacetate (4.0 g, 96%) as a yellow oil.

N-(2-Naphthoyl)-3-aminophenylacetic acid. A portion of the methyl 3-aminophenylacetate (1.65 g, 10 mmol) was dissolved in methylene chloride (50 mL). DIEA (3.5 mL, 20 mmol) then 2-naphthoyl chloride (2.0 g, 10.5 mmol) dissolved in methylene chloride (10 mL) were added to the resulting solution. After 16 hours the reaction was diluted with ethyl acetate (100 mL) and washed with water (50 mL), saturated ammonium chloride (50 mL), and brine (50 mL). The organics were dried over magnesium sulfate and the solvent was removed by rotary evaporation. The resulting solid was recrystallized from refluxing ethyl acetate. Methyl N-(2-naphthoyl)-3-aminophenylacetate (2.1 g, 66%) was obtained as an off-white solid. This was dissolved in methanol (20 mL) and THF (20 mL). A solution of lithium hydroxide (0.84 g, 20 mmol) dissolved in water (15 mL) was added and the reaction was allowed to stir for 4 hours. The solvent was removed by rotary evaporation. The resulting solid was dissolved in water and washed with ethyl acetate (30 mL). The aqueous phase was acidified to pH = 3 with 1M HCl and extracted with ethyl acetate. The organics were washed with brine and dried over magnesium sulfate. The solvent was removed by rotary evaporation. The resulting solid was recrystallized from refluxing ethyl acetate to afford pure N-(2-naphthoyl)-3-aminophenylacetic acid (1.4 g, 70%) as a white solid.

N-(1-Naphthoyl)-3-aminophenylacetic acid. A second portion of methyl 3-aminophenylacetate (1.65 g, 10 mmol) was dissolved in methylene chloride (50 mL). DIEA (3.5 mL, 20 mmol) then 1-naphthoyl chloride (2.0 g, 10.5 mmol) dissolved in methylene chloride (10 mL) was added to the resulting solution. After 16 hours the solvent was removed by rotary evaporation. The resulting solid was dissolved in ethyl acetate (100 mL) and methylene chloride (25 mL) and washed with water (50 mL), saturated ammonium chloride (50 mL) and brine (50 mL). The organic layer was dried over magnesium sulfate and the solvent was removed by rotary evaporation. The resulting solid was recrystallized from refluxing ethyl acetate. Methyl N-(1-naphthoyl)-3-aminophenylacetate (2.2 g, 69%) was obtained as a white solid. This was dissolved in methanol (20 mL) and THF (20 mL). A solution of lithium hydroxide (0.76 g, 18 mmol) was added to the solution and the reaction was allowed to stir for 5 hours. The solvent was removed by rotary evaporation. The resulting solid was dissolved in water (100 mL) and washed with ethyl acetate (30 mL). The aqueous

phase was acidified to pH = 3 with 1M HCl and extracted with ethyl acetate. The organics were washed with brine and dried over magnesium sulfate. The solvent was removed by rotary evaporation. The resulting solid was recrystallized from refluxing ethyl acetate to afford pure N-(1-naphthoyl)-3-aminophenylacetic acid (1.4 g, 68%) as a white solid.

N-(2-Naphthoyl)-2-aminophenylacetic acid and N-(1-naphthoyl)-2-aminophenylactic acid.

Methyl 2-aminophenylacctate. 2-Nitrophenylacetic acid (5.4 g, 30 mmol) was dissolved in methanol (50 mL). Sulfuric acid (1.7 mL, 30 mmol) was added and the reaction was stirred for 16 hours. The solvent was removed by rotary evaporation. The resulting oil was dissolved in ethyl acetate (100 mL) and washed with 10% sodium carbonate (50 mL), brine (50 mL). The organic layer was dried over magnesium sulfate and the solvent was removed to give the methyl ester as a yellow oil (5.2 g, 89%). This was dissolved in methanol (100 mL) and Pearlman's catalyst (50 mg) was added. This was hydrogenated under 50 psi hydrogen for 3 hours. The catalyst was removed by vacuum filtration through a pad of celite. The solvent was removed by rotary evaporation to give methyl 2-aminophenylacetate (4.4 g, 99%) as a colorless oil.

N-(2-Naphthoyl)-2-aminophenylacetic acid. Methyl 2-aminophenylacetate (1.6 g, 10 mmol) was dissolved in methylene chloride (50 mL) and DIEA (3.5 mL, 20 mmol), then 2-naphthoyl chloride (2.0 g, 10.5 mmol) dissolved in methylene chloride (10 mL) were added. After 16 hours the reaction was diluted with ethyl acetate (100 mL) and washed with saturated ammonium chloride (50 mL) and brine (50 mL). The organic layer was dried over magnesium sulfate, and the solvent was removed by rotary evaporation. The crude product was chromatographed on silica gel with methylene chloride to give pure methyl N-(2-naphthoyl)-2-aminophenylacetate (2.3 g, 72%) as a yellow oil that crystallized on standing. This was dissolved in methanol (20 mL) and THF (20 mL) and a solution of lithium hydroxide (0.92 g, 22 mmol) in water (20 mL) was added and stirred for 4 hours. The solvent was removed by rotary evaporation. The resulting solid was dissolved in water (100 mL) and washed with ethyl acetate (3 x 20 mL). The aqueous phase was acidified to pH = 3 with 1 M HCl, and extracted with ethyl acetate. The organics were washed with brine and dried over magnesium sulfate. The solvent was removed by rotary evaporation. The resulting solid was recrystallized from refluxing ethyl acetate to afford pure N-(2-naphthoyl)-2-aminophenylacetic acid (1.15 g, 52%) as a white solid.

N-(1-Naphthoyl)-2-aminophenylactic acid. A second portion of methyl 2-aminophenylacetate (1.65 g, 10 mmol) was dissolve in methylene chloride (50 mL). DIEA (3.5ml, 20 mmol) then 1-naphthoyl chloride (2.0 g, 10.5 mmol) in methylene chloride (10 mL) was added to the solution. After 16 hours the solvent was removed by rotary evaporation. The residue was dissolved in ethyl acetate (100 mL), washed with water (50 mL), saturated ammonium chloride (50 mL), and brine (50 mL). The organic layer was dried over magnesium chloride. The product was recrystallized from refluxing ethyl acetate to give clean methyl N-(1-naphthoyl)-2-aminophenylacetate (2.0 g, 63%) as a pink solid. This was dissolved in methanol (10 mL) and THF (10 mL) and a solution of lithium hydroxide (0.76 g, 18 mmol) in water (15 mL) was added and stirred for 4 hours. The solvent was removed by rotary evaporation. The resulting solid was dissolved in water (100 mL) and washed with ethyl acetate (30 mL). The aqueous phase was acidified to pH = 3 with 1M HCl and extracted with ethyl acetate. The organics were washed with brine and dried over magnesium sulfate. The solvent was removed by rotary evaporation. The resulting solid was recrystallized from refluxing ethyl acetate to afford pure N-(1-naphthoyl)-2-aminophenylacetic acid (1.36 g, 63%) as a white solid.

N-(1-Naphthoyl)-4-aminophenylacetic acid. Methyl 4-aminophenylacetate (1.6 g, 9.6 mmol) was dissolved in methylene chloride (100 mL). DIEA (3.5 mL, 20 mmol) then 1-naphthpyl chloride (1.9 g, 10 mmol) dissolved in methylene chloride (10 mL) were added. After 4 hours the solvent was removed by rotary evaporation. The residue was dissolved in ethyl acetate (100 mL) and saturated ammonium chloride (50 mL). The organic phase was washed with 1 M HCl (20 mL), water (2 x 50 mL), and brine (50 mL) then dried over magnesium sulfate. The solvent was removed by rotary evaporation. The resulting brown solid was recrystallized from refluxing ethyl acetate (the mother liquors were diluted with ether) to afford methyl N-(1-naphthoyl)-4-aminophenylacetate (1.9 g, 63%) as a white solid. This was dissolved in methanol (20 mL) and THF (20 mL) and a solution of lithium hydroxide (0.84 g, 20 mmol) dissolved in water (15 mL) was added and stirred for 16 hours. The solvent was removed by rotary evaporation. The solid was dissolved in water (1000 mL) containing a small amount of sodium carbonate. This was washed with ethyl acetate. The aqueous phase was acidified with 1 M HCl and extracted with ethyl acetate (4 x 250 mL). The organics were washed with brine and dried over magnesium sulfate. The solvent was removed by rotary evaporation. The resulting solid was recrystallized from refluxing ethyl acetate

(1000 mL) to afford N-(1-naphthoyl)-4-aminophenylacetic acid (1.16 g, 67%) as a white solid.

4-(9-Fluorenylmethoxycarbonylamino)phenylacetic acid. A mechanically-stirred suspension of 4-aminophenylacetic acid (10.0 g, 66.2 mmol) in dioxane (100 mL) and 1M aqueous sodium carbonate (165 mL) was cooled to 0 °C and 9-fluorenylmethyl chloroformate (20.54 g, 79.4 mmol) was added. The reaction mixture was allowed to warm to room temperature and was stirred for 16 h. The resulting suspension was acidified to pH = 2 by addition of 12 N hydrochloric acid and the solid product isolated by filtration. The aqueous filtrate was extracted with ethyl acetate (2 x 250 mL). The solid filter cake was dissolved in ethyl acetate (1.5 L) and 0.12 N hydrochloric acid (250 mL) and the phases separated. The ethyl acetate solutions were combined and concentrated *in vacuo* to afford an off white solid. This product was suspended in ethyl acetate (100 mL), isolated by filtration and dried to afford the title compound as a white solid (18.9 g, 50.6 mmol, 76%). ^{1}H NMR ($CDCl_3$): δ 12.28 (s, 1H, CO_2H); 9.69 (s, 1H, NH); 7.91 (d, J = 7.2 Hz, 2H); 7.75 (d, J= 7.2 Hz, 2H); 7.51-7.22 (m, 6H); 7.15 (d, J = 8.1 Hz); 4.48 (d, J= 6.6 Hz, 2H); 4.31 (t, J = 6.6 Hz, 1H); 3.49 (s, 2H).

N-(E)-Cinnamyl-β-alanine. β-Alanine methyl ester hydrochloride (2.79g) was stirred in a solution containing methanol (80 mL) and 0.5N sodium methoxide in methanol (40 mL). The volume was reduced and the reaction mixture was filtered to remove salts. To the filtrate trans-cinnamaldehyde (2.5ml) was added. Upon stirring for 18 h the reaction was cooled to 0 °C and sodium borohydride (1.89 g) was added portion-wise over 2.5 hours. The methanol was removed under reduced pressure and the residue was dissolved in methylene chloride. The organic solution was washed with aqueous sodium bicarbonate and brine then it was concentrated and dried completely on vacuum pump. The crude product was dissolved in THF (50 mL) and a solution of di-t-butyl dicarbonate (17.46 g) and triethylamine (13.9 mL) in THF (60 mL) was added drop-wise. Upon stirring for 18 h the reaction was concentrated and the residue dissolved in ethyl acetate washed with water and brine; concentrated and purified by column chromatography to afford a yellow oil (2.55 g). Hydrolysis of the methyl ester was completed by stirring with sodium hydroxide (1N, 16 mL), water (1.1 mL) and methanol(50 mL) for 5 h. The solution was concentrated under reduced pressure and the remaining aqueous layer was acidified. This was extracted with methylene chloride (3 x 50 mL), concentrated and dried on vacuum pump to afford product, N-cinnamyl-β-alanine (2g).

Assembly Procedure A (compounds where R^8 terminates with $-C(=O)NH_2$)

4-(2-Quinoxaloylamido)phenylacetyl-3-(R)-amido-(2-oxopyrrolidine)-1-α-(1-L-(S)-methylpropyl)acetyl-L-aspartyl-D-argininyl-L-isoleucinylcarboxamide (Procedure A, Method 1):

Coupling of Ile. Rink amide resin (1.5 g) was suspended in DMF (20 mL) and was gently agitated for 30 minutes. The solvent was drained from the resin, 20% (v/v) piperidine in DMF (20 mL) was added, and the suspension was gently agitated for 10 minutes. The piperidine solution was drained from the resin and the resin was washed with DMF (2 x 20 mL). The piperidine treatment was repeated. Following the last DMF wash, the resin was suspended in DMF (5 mL) and N-fluorenylmethyloxycarbonyl-L-isoleucine (0.795 g), HATU (0.813 g) and 1M N,N-diisopropylethylamine in DMF (4.3 mL) were added. The mixture was gently agitated for 3.5 h. The reaction solution was drained from the resin and the resin was washed with DMF (4 x 20 mL). A Kaiser test indicated the presence of free amine, therefore the resin was resuspended in DMF (5 mL) and N-fluorenylmethyloxycarbonyl-L-isoleucine (0.795 g), HATU (0.813 g) and 1M N,N-diisopropylethylamine in DMF (4.3 mL) were added. The mixture was gently agitated for 2.25 h, the reaction solution was drained from the resin and the resin was washed with DMF (4 x 20 mL). The resin was treated with 20% (v/v) piperidine (20 mL) for 10 min, then was washed with DMF (2 x 20 mL). This treatment was repeated, and the resin was washed with additional DMF (2 x 20 mL).

Coupling of D-Arg. The resin was suspended in DMF (5 mL) and N-fluorenylmethyloxycarbonyl-D-Arg(Pbf)-OH (1.39 g), HATU (0.813 g) and 1M N,N-diisopropylethylamine in DMF (4.3 mL) were added. The mixture was gently agitated for 2 h. The reaction solution was drained from the resin and the resin was washed with DMF (4 x 20 mL). The resin was treated with 20% (v/v) piperidine (20 mL) for 10 min, then was washed with DMF (2 x 20 mL). This treatment was repeated, and the resin was washed with additional DMF (2 x 20 mL).

Coupling of Asp. The resin was suspended in DMF (5 mL) and N-fluorenylmethyloxycarbonyl-L-Asp(O-t-Bu)-OH (0.88 g), HATU (0.813 g) and 1M N,N-diisopropylethylamine in DMF (4.3 mL) were added. The mixture was gently agitated for 1.75 h. The reaction solution was drained from the resin and the resin was washed with DMF (4 x 20 mL). The resin was treated with 20% (v/v) piperidine (20 mL) for 10 min, then was

washed with DMF (2 x 20 mL). This treatment was repeated, and the resin was washed with additional DMF (2 x 20 mL).

Coupling of Freidinger lactam. The resin was suspended in DMF (5 mL) and N-fluorenylmethyloxycarbonyl-L-Ile(lactam)-OH (0.932 g), HATU (0.813 g) and 1M N,N-diisopropylethylamine in DMF (4.3 mL) were added. The mixture was gently agitated for 1 h. The reaction solution was drained from the resin and the resin was washed with DMF (4 x 20 mL). The resin was treated with 20% (v/v) piperidine (20 mL) for 10 min, then was washed with DMF (2 x 20 mL). This treatment was repeated, and the resin was washed with additional DMF (2 x 20 mL).

Coupling of 4-aminophenylacetic acid. The resin was suspended in DMF (5 mL) and N-fluorenylmethyloxycarbonyl-4-aminophenylacetic acid (0.798 g), HATU (0.813 g) and 1M N,N-diisopropylethylamine in DMF (4.3 mL) were added. The mixture was gently agitated for 1 h. The reaction solution was drained from the resin and the resin was washed with DMF (4 x 20 mL). The resin was treated with 20% (v/v) piperidine (20 mL) for 10 min, then was washed with DMF (2 x 20 mL). This treatment was repeated, and the resin was washed with additional DMF (2 x 20 mL).

Coupling of 2-quinoxaloyl chloride. The resin was suspended in DMF (5 mL) and 2-quinoxaloyl chloride (0.412 g) and 1M N,N-diisopropylethylamine in DMF (4.3 mL) were added. The mixture was gently agitated for 5.5 h. The reaction solution was drained from the resin and the resin was washed with DMF (4 x 20 mL). A second coupling of 2-quinoxaloyl chloride (0.412 g) and 1M N,N-diisopropylethyl amine in DMF (4.3 mL) was conducted for 12 h. The reaction solution was drained from the resin and the resin was washed with DMF (4 x 20 mL).

The resin was washed with methanol (2 x 20 mL) and diethyl ether (2 x 20 mL) and was then dried *in vacuo*. The product peptide was cleaved from the resin by treatment with trifluoroacetic acid containing 2% (v/v) thioanisole (85 mL) for 4 h. The resin was removed by filtration and washed with trifluoroacetic acid (2 x 10 mL). The filtrate was concentrated to afford a red oil which was triturated with diethyl ether (4 x 100 mL) to afford the crude product as a yellow solid (0.700 g). The product was purified by preparative HPLC on a 45 mm i.d. x 30 cm Dynamax C_{18} 300 Å column using an acetonitrile/water (+0.1% (v/v) trifluoroacetic acid) gradient at a flow rate of 60 mL/min. Fractions containing the desired product were pooled and lyophilized to afford the pure title compound as a pale yellow solid.

Yield: 0.47 g. HPLC (Method f): 6.97 min. MS (ES+): m/z 887.6 Da $(M+H)^+$. ^1H NMR (d_6-DMSO + TFA-d): δ 9.59 (s, 1H), 8.32 (m, 1H), 8.25 (m, 1H), 8.04 (m, 2H), 7.90 (d, J= 8.7 Hz, 2H), 7.35 (d, J= 8.7 Hz, 2H), 4.52 (m, 2H), 4.39 (m, 1H), 4.30 (d, J= 11.1 Hz, 1H), 4.18 (m, 1H), 3.76 (m, 1H), 3.51 (s, 2H), 3.30 (m, 2H), 3.11 (m, 2H), 2.70 (m, 2H), 2.48 (m, 2H), 1.77 (m, 4H), 1.43 (m, 6H), 1.05 (m, 2H), 0.85 (m, 12H).

4-(2-Quinoxaloylamido)phenylacetyl-3(R)-amido-(2-oxopyrrolidine)-1-α-(1-L-(S)-methylpropyl)acetyl-L-aspartyl-D-argininyl-L-isoleucinyl carboxamide (Procedure A, Method 2):

The peptide was assembled by a method analogous to that described in Method 1 up to the coupling of the Freidinger lactam component starting with 0.5 g of RINK amide resin. The N-terminal substituent was then appended:

Coupling of 4-(2-quinoxaloyl)-amidophenylacetic acid. The resin was suspended in DMF (5 mL) and 4-(2-quinoxaloyl)-amidophenylacetic acid (0.23 g), HATU (0.285 g) and 1M N,N-diisopropylethylamine in DMF (1.5 mL) were added. The mixture was gently agitated for 1 h. The reaction solution was drained from the resin and the resin was washed with DMF (4 x 20 mL). The resin was washed with methanol (2 x 20 mL) and diethyl ether (2 x 20 mL) and was then dried *in vacuo*. The product peptide was cleaved from the resin by treatment with trifluoroacetic acid containing 2% (v/v) thioanisole (30 mL) for 3 h. The resin was removed by filtration and washed with trifluoroacetic acid (10 mL). The filtrate was concentrated to afford a red oil which was triturated with diethyl ether (3 x 100 mL) to afford the crude product as a yellow solid (0.184 g). The product was purified on a C_{18} SepPak. Fractions containing the desired product were pooled and lyophilized to afford the title compound as a pale yellow solid. Yield: 0.086 g.

Ethyl 4-(2-quinoxaloyl)amidophenylacetate. A solution of ethyl 4-aminophenylacetate (0.50 g) in dichloromethane (10 mL) was treated with 2-quinoxaloyl chloride (0.51 g) and N,N-diisopropylethylamine (0.38 g). The reaction mixture was stirred at room temperature for 2.5 h, then was diluted with ethyl acetate (50 mL) and washed sequentially with 0.1 N hydrochloric acid (2 x 50 mL), water (50 mL) and brine (50 mL). The aqueous washes were extracted with ethyl acetate (50 mL). The organic extracts were combined, dried over $MgSO_4$, filtered and evaporated to yield the title compound as a yellow solid. Yield 0.81 g. ^1H NMR ($CDCl_3$) δ 9.87 (s, 1H), 9.79 (s, 1H), 8.23 (m, 2H), 7.91 (m,

2H), 7.81 (d, J= 8.1 Hz, 2H), 7.36 (d, J= 8.1 Hz, 2H), 4.17 (q, J= 6.9 Hz, 2H), 3.64 (s, 2H), 1.27 (t, J= 6.9 Hz, 3H). MS (APCI) m/z 358 $(M+Na)^+$, 336 $(M+H)^+$.

4-(2-quinoxaloyl)amidophenylacetic acid. A solution of ethyl 4-(2-quinoxaloyl)amido phenylacetate (0.8 g) and lithium hydroxide monohydrate (0.2 g) in tetrahydrofuran (20 mL), methanol (15 mL) and water (15 mL) was stirred at room temperature for 16 h. The mixture was then diluted with water (20 mL) and concentrated *in vacuo* to a final volume of ca. 40 mL. The solution was acidified to pH = 2 by addition of 1N hydrochloric acid to afford the product as a yellow precipitate, which was isolated by filtration, washed with water (2 x 10 mL) and dried. Yield: 0.68 g. ^{1}H NMR (d_6-DMSO): δ 12.3 (brs, 1H), 10.8 (s, 1H), 9.56 (s, 1H), 8.31 (m, 1H), 8.24 (m, 1H), 8.03 (m, 2H), 7.88 (d, J= 8.4 Hz, 2H), 7.26 (d, J= 8.4 Hz, 2H), 3.58 (s, 2H).

Procedure B (compounds where R^8 terminates with aromatics, cycloalkyls, and heterocycles)

4-(2-Naphthaloylamido)phenylacetyl-3-(R)-amido-(2-oxopyrrolidine)-1-α-(1-L-(S)-methylpropyl)acetyl-L-aspartyl-N-(2-indanoyl)carboxamide (Procedure B, Method 1)

Coupling of Asp. Polystyrene-PEG-PAC resin (50 g, 0.16 meq./gram) was suspended in DMF (300 mL) and was gently agitated for 30 minutes. The solvent was drained from the resin, and the resin was washed with additional DMF (2 x 200 mL). Following the last DMF wash, N-fluorenylmethyloxycarbonyl-L-Asp-α-(allyl)-OH (15.8 g) in DMF (~30 mL), 1,3-diisopropylcarbodiimide (7.52 mL) and 0.08M of 4-dimethylaminopyridine in DMF (10 mL) were added to the resin. The mixture was gently agitated for 4.5 h. The reaction solution was drained from the resin and the resin was washed with DMF (4 x 200 mL). FT-IR analysis showed a strong absorption at 1760 cm^{-1}. To determine if the resin had been fully derivatized, 0.1 g of derivatized resin was treated with N-fluorenylmethyloxycarbonyl-L-Asp-α-(allyl)-OH (0.0316 g.), 1,3-diisopropylcarbodiimide (15 mL) and 0.08M of 4-dimethylaminopyridine in DMF (0.10 mL). The mixture was gently agitated for 3.5 h. The reaction solution was drained from the resin and the resin was washed with DMF (4 x 4 mL). FT-IR analysis showed no change in intensity of the 1760 cm^{-1} absorption indicating that double coupling is unnecessary. The resin was treated with 20% (v/v) piperidine (250 mL) for 10 min, then was washed with DMF (2 x 300 mL). This treatment was repeated, and the resin was washed with additional DMF (4 x 250 mL).

Coupling of Freidinger lactam. A solution of N-fluorenylmethyloxycarbonyl-L-Ile(lactam)-OH (5.24 g), HATU (4.41 g), N,N-diisopropylethylamine (3.3 mL) in DMF (150 mL) was added to the resin. The mixture was gently agitated for 4 h. The reaction solution was drained from the resin and the resin was washed with DMF (4 x 200 mL). A Kaiser test indicated the presence of free amine, therefore the resin was treated with N-fluorenylmethyloxycarbonyl-L-Ile(lactam)-OH (1.45 g), HATU (1.22 g) and N,N-diisopropylethylamine (0.91 mL) in DMF (45 mL). The mixture was gently agitated overnight. The reaction solution was drained from the resin and the resin was washed with DMF (4 x 200 mL). A Kaiser test indicated no free amine. The resin was treated with 20% (v/v) piperidine (250 mL) for 10 min, then was washed with DMF (2 x 300 mL). This treatment was repeated, and the resin was washed with additional DMF (4 x 250 mL).

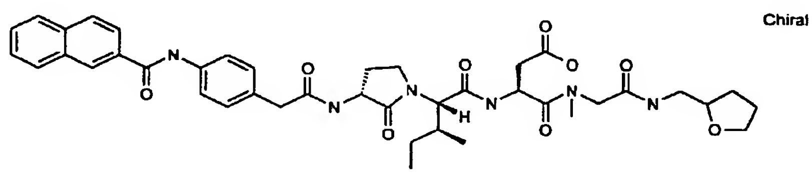
Coupling of 4-(2-naphthaloylamido) phenylacetic Acid. A solution of 4-(2-naphthaloylamido)phenylacetic acid (7.32 g), HATU (8.97 g) and N,N-diisopropylethylamine (6.56 mL) in DMF (150 mL) was added to the resin. The mixture was gently agitated for 4 h. The reaction solution was drained from the resin and the resin was washed with DMF (4 x 200 mL). A Kaiser test indicated no free amine. The reaction solution was drained from the resin and the resin was washed with DMF (4 x 250 mL).

Removal of the allyl protecting group from Asp-α-allyl ester. The resin was washed with: DMF (2 x 300 mL); methylene chloride (5 x 300 mL); and 5% acetic acid / 2.5% N- methylmorpholine in methylene chloride (2 x 350 mL). The washed resin was suspended in 5% acetic acid / 2.5% N-methylmorpholine in methylene chloride(800 mL). Tetrakis (triphenylphosphine)palladium(0) (14 g) was added and mixed gently for 48 hr. The reaction solution was drained from the resin and the resin was washed with: 5% acetic acid / 2.5% N- methylmorpholine in methylene chloride (5 x 350 mL); methylene chloride (3 x 300 mL); 0.5% sodium dietyldithiocarbamate in DMF (4 x 300 mL); DMF (5 x 300 mL); methylene chloride (4 x 300 mL); 10% acetic acid in methylene chloride (4 x 300 mL); and ether (6 x 300 mL). The resin was dried under high vacuum for 18h. Yield : 45 g.

Coupling of 2-aminoindan. A solution of 2-aminoindan hydrochloride (0.271 g), HATU (0.597 g), and N,N-diisopropylethylamine (0.56 mL) in DMF (4 mL) was added to the resin (2.0 g). The mixture was gently agitated for 4 h. The reaction solution was drained from the resin and the resin was washed with DMF (4 x 20 mL). The resin was treated with a solution of 2-aminoindan hydrochloride (0.271 g). HATU (0.597 g), and N,N-

diisopropylethylamine (0.56 mL) in DMF (4 mL). The mixture was gently agitated for 18 hr. The reaction solution was drained from the resin and the resin was washed with DMF (5 x 20 mL), methylene chloride (5 x 20 mL), and diethyl ether (5 x 20 mL) and was then dried *in vacuo*. The product peptide was cleaved from the resin by treatment with trifluoroacetic acid containing 2.5% (v/v) water (35 mL) for 1.5 h. The resin was removed by filtration and washed with trifluoroacetic acid (2 x 10 mL). The filtrate was concentrated to afford a tan oil which was triturated with diethyl ether (3 x 50 mL) to afford the crude product as a white solid (0.200 g). The product was purified by preparative HPLC on a 25 mm i.d. x 20 cm Waters 300 Å column using an acetonitrile/water (+0.1% (v/v) trifluoroacetic acid) gradient at a flow rate of 12 mL/min. Fractions containing the desired product were pooled and lyophilized to afford the pure title compound as a white solid. Yield : 0.083g. MS (API+): 734.

4-(2-Naphthaloylamido)phenylacetyl-3-(R)-amido-(2-oxopyrrolidine)-1-α-(1-L-(S)-methylpropyl)acetyl-L-aspartyl-sarcosinyl-N-[(+/-)-tetrahydrofurfuryl]carboxamide (Procedure B, Method 2):



The peptide was assembled by the method described in Procedure B, method 1. The only difference is that the amine components were synthesized on the Bohdan RAM synthesizer using low temperature conditions.

Coupling of sarcosinyl-N-[(+/-)-tetrahydrofurfuryl]carboxamide.HCl. The following solution containing: sarcosinyl-N-[(+/-)-tetrahydrofurfuryl]carboxamide.HCl (0.125 g), HATU (0.036 g), N,N-diisopropylethylamine (0.054ml) and 1-methylimidazole (0.012ml) in DMF (4 mL) was added to the resin (0.6 g). The mixture was gently agitated for 4 h. The reaction solution was drained from the resin and the resin was washed with DMF (4 x 20 mL). The resin was treated with the following solution containing: sarcosinyl-N-[(+/-)-tetrahydrofurfuryl]-carboxamide.HCl (0.125 g), HATU (0.036 g), N,N-diisopropylethylamine (0.054ml) and 1-methylimidazole (0.012 mL) in DMF (4 mL). The mixture was gently

agitated for 18 hr. The reaction solution was drained from the resin and the resin was washed with DMF (5 x 20 mL), methylene chloride (5 x 20 mL), and diethyl ether (5 x 20 mL) and was then dried *in vacuo*. The product peptide was cleaved from the resin by treatment with trifluoroacetic acid containing 2.5% (v/v) water (35 mL) for 1.5 h. The resin was removed by filtration and washed with trifluoroacetic acid (2 x 10 mL). The filtrate was concentrated to afford a clear oil which was triturated with diethyl ether (3 x 50 mL) to afford the crude product as a white solid (0.096 g).

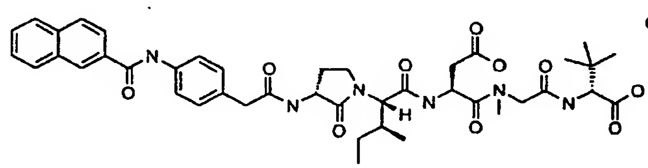
The product was purified by preparative HPLC on a 25 mm i.d. x 20 cm Waters 300 Å column using an acetonitrile/water (+0.1% (v/v) trifluoroacetic acid) gradient at a flow rate of 12 mL/min. Fractions containing the desired product were pooled and lyophilized to afford the pure title compound as a white solid. Yield : 0.060g. HPLC 25.52 min. MS (API-Na+): 793.

Preparation of sarcosinyl-N-[(+/-)-tetrahydrofurfuryl]carboxamide.HCl

t-Boc-sarcosinyl-N-[(+/-)-tetrahydrofurfuryl]carboxamide. In the Bohdan RAM synthesizer using the cooling block reactor, a solution containing t-boc-sarcosine (0.38 g) and N-methylmorpholine (0.242 mL) in THF (6.0 mL) was cooled to -20 °C. To this cooled solution, isobutyl chloroformate (0.262 mL) in THF (3.0 mL) was added using a slow syringe speed. The reaction was kept at -20 °C for two hours with mixing every 30 minutes by way of nitrogen bubbling. A solution containing tetrahydrofurfurylamine (0.248 mL), 1-methylimidazole (0.264 mL) in THF (4.0 mL) was added using a slow syringe speed. The reaction was kept at -20 °C for four hours with mixing every 30 minutes by way of nitrogen bubbling. The cooling unit was then turned off and the reaction was allowed to warm to room temperature overnight with mixing every 30 minutes by way of nitrogen bubbling. The solvent was removed under vacuum. The residue was dissolved in ethyl acetate (75 mL) and washed with water (2 x 10 mL), acetic acid (10% aqueous, 3 x 15 mL), and NaOH (1M, 2 x 10 mL). The organic extract was dried over $MgSO_4$, filtered and evaporated. HPLC 9.5min. MS (API+): 273.

Sarcosinyl-N-[(+/-)-tetrahydrofurfuryl]carboxamide.HCl. t-Boc-sarcosinyl-N-[(+/-)-tetrahydrofurfuryl]carboxamide was dissolved in 6 N HCl (10 mL) and for four hours. The reaction was diluted with water (30 mL), shell-frozen and lyophilized to afford the pure title compound as a white solid. Yield : 0.410g. HPLC 3.5min. MS (API+): 173.

4-(2-Naphthaloylamido)phenylacetyl-3-(R)-amido-(2-oxopyrrolidine)-1-α-(1-L-(S)-methylpropyl)acetyl-L-aspartyl-sarcosinyl-D-tertiary-leucine (Procedure B, Method 3):



The peptide was assembled using the Milligen 9050 continuous flow automated peptide synthesizer by the fmoc/t-butyl strategy on Pepsyn KA(100) resin.

Coupling of D-tertiary leucine. Pepsyn KA(100) resin (5.0 g, 0.10 meq./gram) was suspended in DMF (30 mL) and was gently agitated for 30 minutes. The solvent was drained from the resin, and the resin was washed with additional DMF (2 x 20 mL). Following the last DMF wash, N-fluorenylmethyloxycarbonyl-D-tertiary leucine (0.884 g) in DMF (~4 mL), 1,3-diisopropylcarbodiimide (0.47 mL) and 0.030 g of 4-dimethylaminopyridine in DMF (2 mL) were added. The mixture was gently agitated for 4.5 h. The reaction solution was drained from the resin and the resin was washed with DMF (4 x 20 mL). This derivatized resin was then packed into a 17 mm x 150 mm Omni high pressure borosilicate glass column with PTFE adjustable end piece and attached to the Milligen 9050 automated peptide synthesizer to complete the synthesis.

Coupling of N-fluorenylmethyloxycarbonyl-Sar-OH. On the Milligen 9050, the fmoc protecting group was removed by use of 20% (v/v) piperidine / DMF using a 10 min cycle. The efficiency of the deprotection and coupling cycles were monitored by recording both pre- and post-column UV absorption (~300-350 nm). The following solution containing: N-fmoc-Sar-OH (0.545 g), HATU (0.660 g), N,N-diisopropylethylamine (0.610 mL) in DMF (5.30 mL) was added to the resin using a two- hour coupling cycle. The resin was then treated with 20% (v/v) piperidine / DMF using a 10 min cycle. The UV monitoring indicated satisfactory coupling and deprotection cycles for these steps.

Coupling of N-fluorenylmethyloxycarbonyl-L-Asp(OBu)-OH. A solution of N-fluorenylmethyloxycarbonyl-L-Asp(OBu)-OH (0.720 g), HATU (0.660 g), and N,N-diisopropylethylamine (0.610 mL) in DMF (5.30 mL) was added to the resin using a three-

hour coupling cycle. The resin was then treated with 20% (v/v) piperidine / DMF using a 10 min cycle. The UV monitoring indicated satisfactory coupling and deprotection cycles for these steps.

Coupling of Freidinger lactam. A solution of N-fluorenylmethyloxycarbonyl-L-Ile(lactam)-OH (0.764 g), HATU (0.660 g), and N,N-diisopropylethylamine (0.610 mL) in DMF (5.30 mL) was added to the resin using a three-hour coupling cycle. The resin was then treated with 20% (v/v) piperidine / DMF using a 10 min cycle. The UV monitoring indicated satisfactory coupling and deprotection cycles for these steps.

Coupling of 4-(2-naphthaloylamido)phenylacetic acid. A solution of 4-(2-naphthaloylamido)phenylacetic acid (0.534 g), HATU (0.660 g), and N,N-diisopropylethylamine (0.610 mL) in DMF (5.30 mL) was added to the resin using a three-hour coupling cycle. The UV monitoring indicated satisfactory coupling cycle for this step. The resin was washed with DMF (5 x 20 mL), methylene chloride (5 x 20 mL), and diethyl ether (5 x 20 mL) and was then dried *in vacuo*. The product peptide was cleaved from the resin by treatment with trifluoroacetic acid containing 2.5% (v/v) water (35 mL) for 1.5 h. The resin was removed by filtration and washed with trifluoroacetic acid (2 x 10 mL). The filtrate was concentrated to afford a clear oil which was triturated with diethyl ether (3 x 50 mL) to afford the crude product as a white solid (0.410 g). The product was purified by preparative HPLC on a 25 mm i.d. x 20 cm Waters 300 Å column using an acetonitrile/water (+0.1% (v/v) trifluoroacetic acid) gradient at a flow rate of 12 mL/min. Fractions containing the desired product were pooled and lyophilized to afford the pure title compound as a white solid. Yield : 0.271g. HPLC 21.72 min. MS (LC-ES+): 801.

Procedure C (compounds where R^8 terminates with -NH-alkyl)

4-(2-quinolinylamido)phenylacetyl-3-(R)-amido-(2-oxopyrrolidine)-1-α-(1-L-(S)-methylpropyl)acetyl-L-aspartyl-D-argininyl-isobutyl

Coupling of isobutylamine. Polystyrene resin with the Ellman's aldehyde linker (50 mmol) was swelled in DMF (1000 mL) and acetic acid (10 mL) for 10 minutes. Isobutylamine (32 mL, 325 mmol) and sodium triacetoxyborohydride (69.5 g, 328 mmol) were added. The mixture was stirred with an overhead stirrer for two hours, then transferred to a fritted glass funnel and was washed with a one to one mixture of methanol and DMF (3 x 300 mL), DMF (3 x 300 mL), methylene chloride (5 x 300 mL) and methanol (5 x 300 mL). The resin was

dried *in vacuo* at 40 °C for 16 hours. MAS-NMR showed disappearance of the aldehyde proton.

Coupling of D-Arg. A portion of this material (8 g, 6.5 mmol) was swelled in DMF for 15 minutes, then added a solution of N-fluorenylmethyloxycarbonyl-D-Arg(Pbf)-OH (5 g, 7.5 mmol), HATU (2.8 g, 7.4 mmol), and DIEA (2.7 mL, 15 mmol) in DMF (50 mL). The reaction was mixed with a gentle nitrogen gas flow for two hours. The liquid was drained and the resin was washed with DMF (10 x 50 mL). NPIT test showed unreacted amine. The resin was treated with a second batch of the reaction cocktail (4.2 g N-fluorenylmethyloxycarbonyl-D-Arg(Pbf)-OH, 2.3 g HATU, and 2.7 mL DIEA in 50 mL DMF) for an additional hour. The liquid was drained and the resin was washed with DMF (10 x 50 mL). NPIT test showed the reaction was complete. The resin was treated with 20% piperidine in DMF (2 x 50 mL) then washed with DMF (10 x 50 mL).

Coupling of Asp. A solution of N-fluorenylmethyloxycarbonyl-L-Asp(O-t-Bu)-OH (6.2 g, 15 mmol), HATU (5.0 g, 13 mmol) and DIEA (5 mL, 30 mmol) in DMF (50 mL) was added to the resin and mixed with a gentle nitrogen gas flow for 16 hours. The liquid was drained and the resin was washed with DMF (10 x 50 mL). Kaiser test showed the reaction was complete. The resin was treated with 20% piperidine in DMF (2 x 50 mL) then washed with DMF (10 x 50 mL).

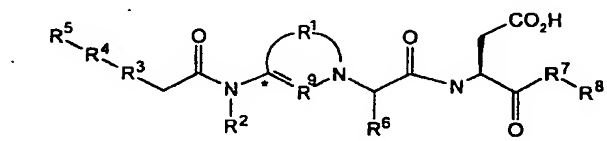
Coupling of Freidinger lactam. A solution of N-fluorenylmethyloxycarbonyl-D-freidingerlactam-L-isoleucine (6.6 g, 15 mmol), HATU (5.0 g, 13 mmol) and DIEA (5.0 mL, 30 mmol) in DMF (50 mL) was added to the resin and mixed with a gentle nitrogen flow for 3 hours. The liquid was drained and the resin was washed with DMF (10 x 50 mL). Kaiser test showed the reaction was complete. The resin was treated with 20% piperidine in DMF (2 x 50 mL) then washed with DMF (10 x 50 mL).

Coupling of 4-aminophenylacetic acid. A solution of N-fluorenylmethyloxycarbonyl-4-aminophenylacetic acid (4.1 g, 11 mmol), HATU (3.8 g, 10 mmol) and DIEA (2.6 mL, 20 mmol) in DMF (50 mL) was added to the resin and mixed with a gentle flow of nitrogen gas for 16 hours. The liquid was drained and the resin was washed with DMF (10 x 50 mL). Kaiser test showed the reaction was complete. The resin was washed with methylene chloride (5 x 50 mL) and ether (5 x 50 mL) then dried *in vacuo* at 40 °C for 5 hours. A portion of this resin (2.4 g, 1 mmol) was swelled in DMF (20 mL) for 30 minutes, treated with 20% piperidine in DMF (2 x 20 mL) then washed with DMF (10 x 20 mL).

Coupling of quinaldic acid. A solution of quinaldic acid (0.52 g, 3 mmol), HATU (0.95 g, 2.5 mmol) and DIEA (1 mL, 6 mmol) in DMF (20 mL) was added to the resin and mixed with a gentle flow of nitrogen gas for 16 hours. The liquid was drained and the resin was washed with DMF (10 x 20 mL), methylene chloride (5 x 20 mL) and ether (5 x 20 mL) then dried *in vacuo* at 40 °C for 30 minutes. The resin was treated with a solution of TFA (50 mL), water (1 mL), thioanisole (1 mL), and TIS (0.5 mL) for one hour. The liquid was filtered from the resin and reduced to about 5 mL by rotary evaporation. Ether (200 mL) was added to precipitate the crude product. Purified by preparative HPLC on C_{18} Dynamax column (21.4 mm x 25 cm, 60A) using a gradient of 20% to 40% acetonitrile in water with 0.5% TFA. Fractions containing pure product were combined and lyophilized to give 0.40 g (42%) of the final product as a yellow solid. HPLC: 93% purity 6.51 min C_{18} Dynamax (5 cm x 4.6 mm, 3 µm particle, 100 Å pore) 20 - 60% acetonitrile/ water (each containing 0.5% TFA) over 7.5 min. at 1 mL/min. MS electrospray M+ 829.4 parent, 415.4 base. HNMR 250 MHz (DMSO-d_6, TFA-d_1) 0.86 m 12H, 0.94 m 2H, 1.48 m 4H, 1.78 m 3H, 1.94 m 1H, 2.32 m 1H, 2.60 m 2H, 2.91 d *J* = 5.5hz 2H, 3.11 t J=5.3hz 2H, 3.35 m 2H, 3.52 s 2H, 4.21 m 1H, 4.26 d *J*=23.3hz 1H, 4.48 m 2H, 7.36 d *J*=7.0 2H, 7.78 t *J*=6.0 1H, 7.92 m 2H, 8.14 d *J*=6.5 1H, 8.30 d *J*=7.0 2H 8.66 d *J*=7.0 1H

Claims:

1. A linear peptide of the formula:



wherein:

R^1 is $-CH_2CH_2-$, $-CH_2CH_2CH_2-$, $=CH-CH=CH-$ or $-N=CH-$;

R^2 is H or CH_3;

R^3 is $-CH_2CH_2CH_2-$, -(E)-CH=CHC(=O)NH-, $-CH_2CH_2C(=O)NH-$, para-disubstituted phenyl, ortho-disubstituted phenyl, meta-disubstituted phenyl or a single bond;

R^4 is -NHC(=O)-, -C(=O)NH- or $-S(=O)_2NH-$;

R^5 is 1-naphthyl, 2-naphthyl, $-CH_2CH_2NHCH_2CH=CH$-phenyl, $-CH_2CH_2$-phenyl, -CH=CH-phenyl, 2-quinolyl, 3-quinolyl, 4-quinolyl, 6-quinolyl, 3-isoquinolyl, 2-quinoxaline, 5-chloro-2-indolyl, 2-indolyl, 4-chlorophenyl, 4-methylphenyl, 3-methoxyphenyl, 4-cyanophenyl, 3,4-difluorophenyl, 3-chloro-4-fluorophenyl, 2,4-dichlorophenyl, 3,4-dichlorophenyl, 4-chlorophenyl, 3,5-dimethoxyphenyl, 4-*tert*-butylphenyl, phenyl, 4-trifluoromethylphenyl, $-CH_2CH_2CH_2$-phenyl, 6-quinolyl-C(=O)-, 2-quinoxaline-C(=O)-, 5-chloro-2-benimidazolyl, fluorenylmethoxycarbonyl, 4-chlorobenzyl, 4-methylbenzyl, 3-quinoxalinyl, 3,4-difluorophenyl, or 4-fluorophenyl;

R^6 is isobutyl or *sec*-butyl;

R^7 is N-methylglycine, $-NHCH_2CH_2NHC(=O)-$, L-arginine, D-arginine, L-ornithine, D-ornithine, histidine, citrulline, proline, hydroxyproline, 3-pyridinylalanine, L-N-methylalanine, D-N-methylalanine, aminobutyric acid, N-2-indolizidinyl or thiazolidine;

R^8 is L-isoleucine-NH_2, D-isoleucine-NH_2, $-CH_2$-cyclopentyl, $-CH_2$-2-tetrahydrofuranyl, tert-butylglycine-NH_2, n-butyl, isobutyl, -NH-cyclopentyl, $-NHCH_2$-2-furanyl, $-NHCH_2$-pyrininyl, $-NHCH_2$-cyclohexyl, D-leucinol, -NH-isobutyl, L-allo-isoleucine-NH_2, 1-hydroxycycloleucinol, 2-(aminomethyl)-1-ethyl-pyrrolidine, or (S)-NH-2-methylbutyl, or R^8 is absent when R^7 is N-2-indolizidinyl;

R^9 is =CH- or -C(=O)-; and

represents a double bond when R^9 is =CH- and a single bond when R^9 is -C(=O)-.

2. The compound of Claim 1, wherein
R^1 is -N=CH-; and
R^9 is =CH-.

3. The compound of Claim 1, wherein:
R^1 is $-CH_2CH_2-$, $-CH_2CH_2CH_2-$ or =CH-CH=CH-; and
R^9 is -C(=O)-.

4. The compound of Claim 3, wherein
R^1 is $-CH_2CH_2-$;
R^3 is para-disubstituted phenyl;
R^4 is -C(=O)NH-;
R^5 is 1-naphthyl, 2-naphthyl, $-CH_2CH_2NHCH_2CH$=CH-phenyl, $-CH_2CH_2$-phenyl, -CH=CH-phenyl, 2-quinolyl, 3-quinolyl, 4-quinolyl, 6-quinolyl, 3-isoquinolyl, 2-quinoxaline, 5-chloro-2-indolyl, 2-indolyl, 4-chlorophenyl, 4-methylphenyl, 3-methoxyphenyl, 4-cyanophenyl, 3,4-difluorophenyl, 3-chloro-4-fluorophenyl, 2,4-dichlorophenyl, 3,4-dichlorophenyl, 4-chlorophenyl, 3,5-dimethoxyphenyl, 4-*tert*-butylphenyl, phenyl or 4-trifluorophenyl; and
R^6 is *sec*-butyl.

5. The compound as recited in Claim 4, wherein
R^2 is H;
R^5 is 2-naphthyl;
R^7 is L-arginine or D-arginine; and
R^8 is isobutyl.

6. The compound of Claim 4, wherein
R^2 is H;
R^5 is 2-quinoxaline;
R^7 is L-arginine or D-arginine; and
R^8 is L-isoleucine-NH_2 or D-isoleucine-NH_2.

7. A pharmaceutical composition having natriuretic, diuretic or vasodilator activity in mammals, comprising a pharmaceutically effective amount of a linear peptide of Claim 1.

8. A method for treating one or more conditions selected from the group consisting of pulmonary hypertension, congestive heart failure, nephritic syndrome, hepatic cirrhosis, pulmonary disease, pulmonary hypertension and renal failure, comprising the step of administering a pharmaceutically-effective amount of a compound according to Claim 1.

9. A method for treating one or more conditions selected from the group consisting of pulmonary hypertension, congestive heart failure, nephritic syndrome, hepatic cirrhosis, pulmonary disease, pulmonary hypertension and renal failure, comprising the step of administering a pharmaceutically-effective amount of a compound according to Claim 4.

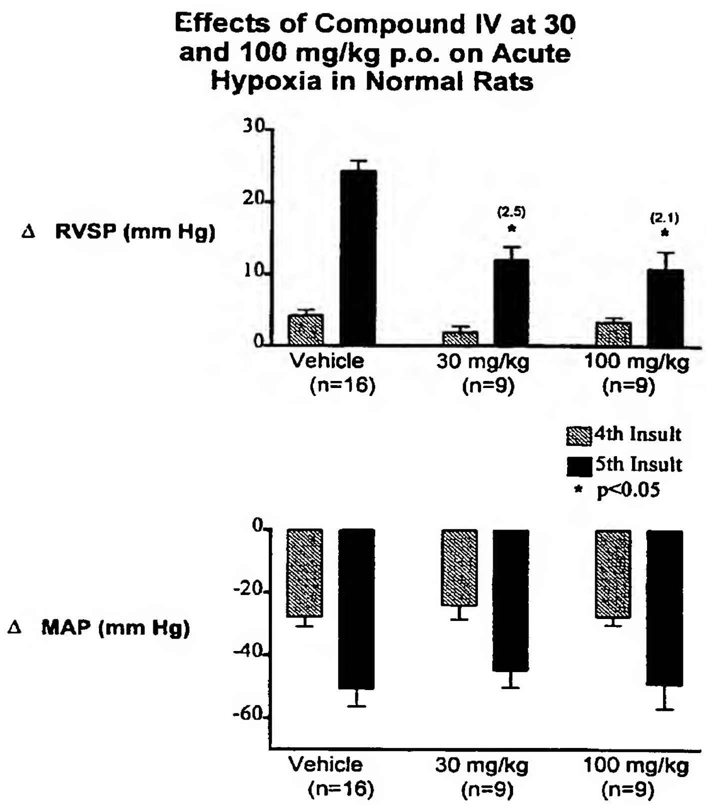
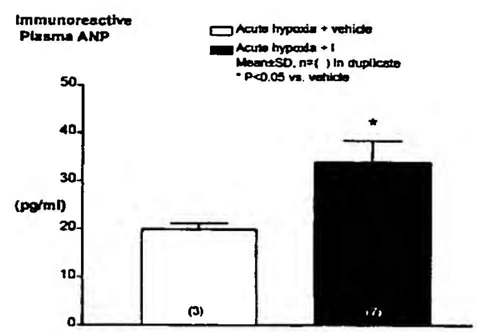

Effects of Compound IV at 30
and 100 mg/kg p.o. on Acute
Hypoxia in Normal Rats
Δ RVSP (mm Hg)
30
20
10
0
(2.5)
*
(2.1)
*
Vehicle
(n=16)
30 mg/kg
(n=9)
100 mg/kg
(n=9)
4th Insult
5th Insult
* p<0.05
Δ MAP (mm Hg)
0
-20
-40
-60
Vehicle
(n=16)
30 mg/kg
(n=9)
100 mg/kg
(n=9)

Figure 1

Effects of I (30mg/kg, p.o.) on
Immunoreactive ANP Plasma Levels in Rats



Effects of IV (100mg/kg, p.o.) on
Immunoreactive ANP Plasma Levels in Rats

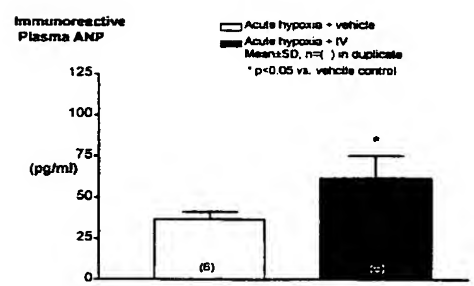


Figure 2

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/GB 00/01319

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/58 A61K38/22 A61P9/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, CHEM ABS Data, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 323 740 A (CALIFORNIA BIOTECHNOLOGY INC) 12 July 1989 (1989-07-12)	
A	EP 0 356 124 A (CALIFORNIA BIOTECHNOLOGY INC) 28 February 1990 (1990-02-28)	
A	DE 42 42 946 A (BAYER AG) 23 June 1994 (1994-06-23)	
A	KOYAMA S ET AL: "AP-811, A NOVEL ANP-C RECEPTOR SELECTIVE AGONIST" INTERNATIONAL JOURNAL OF PEPTIDE AND PROTEIN RESEARCH, DK, MUNKSGAARD, COPENHAGEN, vol. 43, no. 4, 1 April 1994 (1994-04-01), pages 332-336, XP000434523 ISSN: 0367-8377	

[ ] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 July 2000	21/07/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Cervigni, S

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 00/01319

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